Motor Functioning and Parkinson's Disease:

Insights from the general population

Sirwan K.L. Darweesh

Acknowledgments:
Financial support for the publication of this thesis by Erasmus MC and the Department of Epidemiology (Erasmus MC) is gratefully acknowledged.

ISBN: 978-94-6323-632-4

Cover design: Sirwan K.L. Darweesh and Khalid L. Darweesh. Depiction of a patient with Parkinson's Disease in the general population.

The illustration of the patient with Parkinson's Disease was originally drawn by Dr. William Gowers. Source of original illustration: Paralysis agitans. A Manual of diseases of the nervous system. Vol. II. Philadelphia: P Blakiston; 1893. p. 636–57. The illustration is free of restrictions under copyright law.

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Layout: Sirwan K.L. Darweesh

Printing: Gildeprint BV, Enschede

Motor Functioning and Parkinson's Disease:
Insights from the general population

Motor functioneren en de ziekte van Parkinson:
inzichten uit de algemene bevolking

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. R.C.M.E. Engels

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

woensdag 29 mei 2019 om 9:30 uur

door

Sirwan Khalid Lefta Darweesh

geboren te Gouda

Erasmus University Rotterdam

PROMOTIECOMMISSIE

Promotoren:	Prof.dr. M.A. Ikram Prof.dr. P.J. Koudstaal
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To my family

TABLE OF CONTENTS

MANUSCRIPTS BASED ON THIS THESIS

Chapter 2.1 **Darweesh SKL**, Adams HHH, Van der Geest JN, Ikram MK, Ikram MA. Parkinson Disease genes, gait and cognition. *Submitted*.

Chapter 2.2 **Darweesh SKL**, Roshchupkin GV, Licher S, Van der Geest JN, Ikram MK, Ikram MA. Microstructural brain determinants of motor functioning. *In preparation*.

Chapter 2.3 Sedaghat S, **Darweesh SKL**, Verlinden VJA, van der Geest JN, Dehghan A, Franco OH, Hoorn EJ, Ikram MA. Kidney function, gait pattern and fall in the general population: a cohort study. *Nephrol Dial Transplant*. 2018;33:2165-72.

Chapter 3.1 Licher S*, **Darweesh SKL***, Wolters FJ*, Fani L, Heshmatollah A, Mutlu U, Koudstaal PJ, Heeringa J, Leening MJG, Ikram MK, Ikram MA. Lifetime risk of common neurological diseases in the elderly population. *J Neurol Neurosurg Psychiatry*. 2019;90:148-56.

Chapter 3.2 **Darweesh SKL**, Wolters FJ, Hofman A, Stricker BH, Koudstaal PJ, Ikram MA. Simple Test of Manual Dexterity Can Help to Identify Persons at High Risk for Neurodegenerative Diseases in the Community. *J Gerontol A Biol Sci Med Sci*. 2017;72:75-81.

Chapter 3.3 **Darweesh SKL**, Licher S, Wolters FJ, Koudstaal PJ, Ikram MK, Ikram MA. Gait, cognitive decline and dementia: the Rotterdam Study. *Alzheimer's&Dementia*. 2019.

Chapter 3.4 **Darweesh SKL**, Wolters FJ, Cremers LG, Hofman A, Koudstaal PJ, Vernooij MW, Ikram MK, Bos D, Vernooij MW, Ikram MA. Motoric cognitive risk syndrome: risk factors, MRI determinants, and prognosis in a community-based cohort. *In preparation*.

Content

Chapter 7.1	**Darweesh SKL**, Verlinden VJ, Stricker BH, Hofman A, Koudstaal PJ, Ikram MA. Trajectories of prediagnostic functioning in Parkinson's disease. *Brain*. 2017;140:429-41.
Chapter 7.2	**Darweesh SKL**, Wolters FJ, Postuma RB, Stricker BH, Hofman A, Koudstaal PJ, Ikram MK, Ikram MA. Association Between Poor Cognitive Functioning and Risk of Incident Parkinsonism: The Rotterdam Study. *JAMA Neurology*. 2017;74:1431-8.
Chapter 7.3	Lysen TS, **Darweesh SKL**, Ikram MK, Luik AI, Ikram MA. Sleep and risk of parkinsonism and Parkinson's disease: a population-based study. *Brain*. 2019.
Chapter 8	**Darweesh SKL**, Koudstaal PJ, Ikram MK, Ikram MA. Cognitive decline before diagnosis of Parkinson's disease. *Lancet Neurol*. 2017;16:262. **Darweesh SKL**, Koudstaal PJ, Ikram MA. Trends in the Incidence of Parkinson's Disease. *JAMA Neurol*. 2016;73:1497. **Darweesh SKL**, Verlinden VJ, Ikram MA. Dual-Task Gait and Incident Dementia: A Step Forward, but Not There Yet. *JAMA Neurol*. 2017;74:1380.

**These authors contributed equally. Note: for manuscripts that have been published, supplementary material is available on the website of the publisher. Those tables or figures are referred to as 'Online Supplementary' material throughout this thesis.*

Chapter 1

General introduction

The disease is of long duration: to connect, therefore, the symptoms which occur in its later stages with those which mark its commencement, requires a continuance of observation of the same case, or at least a correct history of its symptoms, even for several years.

James Parkinson. An Essay on the Shaking Palsy; Preface. 1817.

Chapter 1

CONTEXT AND KEY GAPS IN KNOWLEDGE

Adequate motor functioning is an essential prerequisite in our daily activities, enabling us to turn off the alarm clock in the morning, get up out of bed, take a shower, and perform other basic and instrumental tasks throughout the day. Motor functioning strongly impacts the ability to maintain functional independence and serves as a useful predictor of adverse health outcomes, in particular among the elderly.[1-3] As the number of elderly individuals is expected to grow due to ageing of populations worldwide, there is now a growing sense of urgency to unravel determinants of motor functioning.

The detrimental influence of motor impairments on functional independence is painfully noticeable in individuals with neurodegenerative movement disorders. Parkinson's Disease (PD) is the most widely recognized among these disorders, currently affecting over 6 million individuals worldwide.[4] PD is primarily characterized by parkinsonism, a clinical syndrome of motor impairments defined by the presence of brady- or hypokinesia in combination with at least one of the following: resting tremor, rigidity or postural instability. PD was first described by James Parkinson in 1817,[5] and his seminal essay marked the beginning of a quest for therapies that could cure or at least slow PD. However, no effective disease-modifying therapies have been identified to date, and the global burden of PD has more than doubled over the last three decades in large part as a result of increasing numbers of elderly individuals.[4] The social and economic burden caused by PD is broadly expected to rise further in the coming decades, although it is noteworthy that such projections assume that the incidence and associated mortality of PD remain stable over time.[6] To better inform future projections on the burden caused by PD, there is a need for empirical data on temporal trends of PD as well as on determinants that drive temporal trends.

Intriguingly, deterioration of motor functioning begins well before individuals are clinically diagnosed with PD, as a result of accumulating pathology in the brain. By the time individuals are clinically diagnosed with PD, over 60% of nerve cells in the nigrostriatal pathway are already depleted in patients with PD. The advanced stage of pathology likely contributes to the failure of trials aimed at effectively modifying disease progression in patients with a clinical diagnosis of PD.[7] As a consequence, there is rapidly increasing interest in the phase before clinical diagnosis. This period is known as the 'prediagnostic' phase of PD, and may span over several years or even decades.[8] From an etiologic perspective, the identification of risk factors (both genetic and non-genetic) and prodromal features of PD may help to unravel underlying mechanisms leading to clinical PD, possibly resulting in novel targets for intervention. From a predictive perspective, the identification of determinants of PD may help to uncover individuals at high risk of PD, which in turn may open the door to early symptomatic treatment and possible inclusion in neuroprotective trials.

Progressive motor impairments may also occur in the prediagnostic phase of other neurodegenerative diseases than PD, including those that are primarily characterized by dementia (e.g., Alzheimer's Disease). Furthermore, motor impairments are often accompanied by cognitive deficits across neurodegenerative diseases, typically adding to the loss of functional independence. However, there is limited empirical evidence on overlap of cognitive and motor impairments in the prediagnostic phase of neurodegenerative diseases or on overlap in their lifetime risk. Taken together, these gaps in knowledge emphasize that an improved understanding of determinants of motor impairments may have implications not only for PD, but also for other neurodegenerative diseases.

Against that background, the overall aim of this thesis is to obtain novel insight on determinants of motor functioning and the prediagnostic phase of PD.

OVERALL APPROACH

In studies aiming to unravel determinants of motor functioning and the prediagnostic phase of PD, it is important to consider how two key features of design can influence a study's findings: setting and timing.

As for the setting, studies can either comprise a sample of individuals at high risk of PD, e.g. those with rare risk-increasing genetic variants implicated in PD (e.g. *LRKK2* mutation carriers) or a sample of individuals that are included irrespective of PD risk. While the former approach generally requires fewer study participants (as the proportion of eventual clinical PD cases is likely higher), the generalizability of findings in such studies to the broad spectrum of PD may be limited if the underlying mechanisms of PD with the high-risk trait and PD without the high-risk trait differ. By contrast, this issue does not substantially affect cohorts of individuals that are included irrespective of PD risk, such as population-based studies.

As for timing, it is important to consider the relationship between assessment of determinants, features and diagnosis of clinical PD. In prospective cohort studies, these assessments take place before diagnosis of clinical PD, and assessments are identical in individuals who are eventually be diagnosed with PD and in others. In retrospective cohort studies, these assessments take place after diagnosis of clinical PD, indicating that information on the exposure to determinants may be

flawed, and assessments may have been different in individuals who are eventually be diagnosed with PD and in others.

Taking into account these considerations on study design, I used a population-based setting to study the key gaps in knowledge outlined above, and applied a prospective approach in studies on prediagnostic PD. In the next paragraphs, I provide a rationale for the specific aims addressed in each chapter of this thesis.

CHAPTER 2

Our understanding of the genetic, cerebral microstructural, and metabolic determinants of specific aspects of gait (e.g., variability in gait pattern, turning ability) remains relatively limited. In **Chapter 2.1**, I focus on the genetic underpinnings of gait and cognitive functioning, which are both hallmarks of neurodegenerative diseases and each have a substantial heritable component. Interestingly, gait or cognitive of gait that are commonly affected in individuals with PD stand out in particular; for instance, over half (60%) of variance in the step-to-step variability of gait patterns may be explained by genetic predisposition.[9] This suggests that genetics provide a unique window of opportunity to unravel causal, overlapping mechanisms across gait, cognition and PD. I hypothesized that genetic variants of PD may evoke (subtle) gait and cognitive deficits in individuals who are (still) free of a clinical neurodegenerative disease. In **Chapter 2.2**, I focus on another group of determinants of motor functioning: markers of cerebral microstructure. Macrostructural lesions in the brain are a well-recognized cause of motor impairments, however, there is substantial variability in motor function performance among individuals without such deficits. Variability in cerebral microstructure may account for some of the

variability in motor function performance, but empirical data on this topic remains scarce. This is a critical gap in available evidence, since it maintains the wide-spread notion that impairment in motor functions is an inevitable consequence of advancing age, possibly delaying clinical care seeking and precluding optimal secondary prevention of further motor decline. In this chapter, I address this gap by assessing associations between cerebral microstructure and motor function performance. In **Chapter 2.3**, I assess the role of metabolism in motor functioning. In particular, I build on the previous observation that chronic kidney failure can have a major influence on gait[10] by studying the effects on gait and falling of subclinical impairment in kidney function, which is highly prevalent in the general population.

CHAPTER 3

In **Chapter 3**, I examine the overlap of cognitive and motor impairments in individuals with (prediagnostic) PD or other neurodegenerative diseases. The burden on the population posed by these diseases may be higher than previously estimated in prevalence studies, since prevalence is not only influenced by incidence of a disease but also by mortality. Lifetime risk estimates take into account incidence as well as mortality and could therefore more accurately inform the general public of the burden posed by a disease. PD patients have an increased susceptibility for dementia and possibly also for stroke,[11,12] yet, empirical data on the overlap in lifetime risk of these syndromes is scarce. This is a critical gap, since lifetime risk estimates could inform the design of trials aimed at preventing these syndromes simultaneously (e.g., through lifestyle

interventions). Therefore, I investigate the lifetime risk of parkinsonism, dementia and stroke in **Chapter 3.1**.

Given the ageing of populations, the burden of age-related impairments in motor functioning and cognitive functioning are expected to increase dramatically in the coming decades. Understanding these impairments, and the relationships between impairments in motor and cognitive functioning, may have broad public health implications and may also elucidate underlying mechanisms, which may lead to novel therapies.[13] Interestingly, deterioration of motor function performance commonly occurs in neurodegenerative diseases that are primarily characterized by dementia, such as Alzheimer's Disease and vascular dementia. In particular, impairments in manual dexterity and gait have emerged as prodromal features of Alzheimer's Disease and vascular dementia.[13-15] As complex traits that require integration of motor and cognitive skills (as well as influences not mediated by the brain), these features embody the phenotypical overlap of neurodegenerative diseases.[16-18] However, it remains unclear whether prediagnostic pathology to the brain can also lead to impaired manual dexterity in individuals who are in the prediagnostic phase of Alzheimer's Disease, vascular dementia or other primary dementia diseases. If so, assessment of these motor functions might contribute to prediction of neurodegenerative diseases in adults who do not have overt cognitive dysfunction (yet). At that stage, pathological processes are typically less advanced in individuals prone to develop dementia, and putative neuroprotective interventions may still have substantial effects on the delay of dementia onset. Against that background, I investigate the associations of manual dexterity (**Chapter 3.2**), gait (**Chapter 3.3**) and the combination of subjective cognitive complaints and subtle objective motor deficits (**Chapter 3.4**) with dementia.

CHAPTER 4

Although PD can affect young individuals, it is especially common among individuals aged 65 years or older.[19] As elderly populations increase, the burden caused by PD (and related diseases characterized by parkinsonism) is generally expected to increase rapidly in the coming decades.[20,21] One key assumption for this projection is that the incidence of PD does not drop, but empirical data on temporal trends of the incidence of PD are scarce. To obtain insight into the future burden of PD, I investigate trends in the incidence of PD and trends in the prevalence of risk factors that may drive these trends in **Chapter 4.1**, and temporal trends in mortality associated with PD in **Chapter 4.2**.

CHAPTER 5

The identification of determinants of PD may improve our understanding of the mechanisms that lead to clinical PD, possibly uncovering targets for intervention. Against that background, genetic variants form a particularly promising group of determinants of PD, since genotypes do not change over time. As a consequence, any robust association between a genetic variant and PD suggests that the variant is either causal for PD or tags another variant that is causal for PD. In recent years, genome-wide association studies have identified tens of common genetic risk variants implicated in PD.[22,23] However, the clinical usefulness of these variants in predicting PD remains untested. Also, it is unclear whether these risk variants evoke symptoms related to PD in individuals without clinical parkinsonism, leading to subtle problems in daily functioning. I investigate these important gaps of knowledge on previously identified genetic variants of PD in **Chapter 5.1**. Of note, most previously identified genetic variants of PD are mapped to non-coding

regions of the genome. By contrast, the role of genetic variants that regulate gene expression remains largely unclear. A particularly promising group of genetic variants are those in microRNAs or miRNA binding sites, since microRNAs serve as key regulators of gene expression.[24,25] MicroRNAs have been shown to be involved in a wide-range of pathogenic processes, and evidence from animal models and case series suggests that dysregulated miRNAs are associated with PD.[26-28] However, the genetic underpinnings of these associations remain unclear. In **Chapter 5.2**, I address this gap by systematically examining the association of variants in miRNAs or miRNA binding sites with PD.

CHAPTER 6

Another promising group of determinants of PD are non-motor determinants, including various putative risk or protective factors. Several non-motor determinants of PD have been identified in previous studies,[29] ranging from addictive behaviour (e.g., smoking and caffeine intake) to use of several cardiovascular medications (e.g., calcium-channel blockers, beta-blockers) and others. However, it remains unclear whether a combination of these determinants can be used to predict PD in the community. This is the focus of attention in **Chapter 6.1**. In addition to these previously established determinants, I also consider two novel classes of non-motor determinants of PD: vascular disease and professional occupation in mid-life. While use of several cardiovascular medications is associated with the risk of PD, vascular disease may itself be associated with PD (in an opposite direction). Previous studies have shown no evidence for an association between clinical vascular diseases (e.g., stroke) and the risk of PD. However, the association between subclinical vascular disease and

PD remains unknown. In **Chapter 6.2**, I assess the association between measures of subclinical vascular disease and PD. Another potentially useful clue on the etiology of PD is that creativity in PD is strongly related to dopaminergic activity and medication. I hypothesized that PD patients, including those who are in the prediagnostic phase of PD, are prone to choose highly-structured 'conventional' professional occupations and avoid highly-creative 'artistic' occupations, and investigate this in **Chapter 6.3**.

CHAPTER 7

At the time of clinical diagnosis, patients with PD already have a wide range of motor and non-motor features that affect their daily functioning. However, the temporal sequence of occurrence of these features remains largely unknown. Insight into such prediagnostic trajectories, and their combined effects on daily functioning, could possibly aid in earlier diagnosis of PD and contribute to the identification of individuals who would benefit from early symptomatic treatment. Moreover, it may inform clinical studies on which individuals may be most suitable for inclusion in neuroprotective trials. Against that background, I investigate patterns of deterioration in motor and non-motor features as well as their combined influence on functional independence during the prediagnostic phase of PD in **Chapter 7.1**.

Although PD is primarily characterized by parkinsonism, dysfunction in a diverse array of cognitive functions is a common feature among patients with PD.[30-33] Interestingly, cognitive functioning has also been reported worse in individuals who are free of parkinsonism but have impaired olfaction and reduced dopamine transporter binding, both of which are strong proxies of PD.[34] Yet, there is a

scarcity of data on cognitive functioning of PD patients before clinical diagnosis. It is also unclear how cognitive deficits combine with subtle motor signs in prediagnostic PD patients. These gaps of knowledge are the focus of attention in **Chapter 7.2**.

In addition to these features, impairments in sleep quality and duration are highly common during the clinical phase of PD, but aside from REM behavior sleep disorder (RBD) there is a scarcity of empirical data on sleep problems in prodromal PD patients. RBD is only present in a minority of patients with prodromal PD,[35] and may therefore merely represent the 'tip of the iceberg' of sleep-wake disturbances in these patients. If that were the case, sleep quality and duration may harbor incremental predictive utility for PD. To address this issue, I investigate the associations of sleep quality and duration with PD in **Chapter 7.3**.

CHAPTER 8

In **Chapter 8**, I integrate the observations described in this thesis in a broader clinical and methodological context, and offer my perspective on future directions of the field.

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Chapter 2

Motor functioning: determinants

The patient often walks with short quick steps, leaning forward as if about to run.

Gowers WR. Paralysis agitans. In: Gowers WR, editor. A Manual of diseases of the nervous system. Vol. II. Philadelphia: P Blakiston; 1893. p. 636–57.

Chapter 2.1

Genetics

ABSTRACT

Subtle cognitive deficits and gait impairments are common prodromal signs of Parkinson's Disease (PD), and their combined presence conveys a much higher risk of clinical PD than either feature in isolation. We hypothesized that genetic variants implicated in PD are associated with worse cognitive functioning and gait. We also hypothesized that genetic associations with cognitive functioning would be more distinct in individuals with below-average gait, and vice versa. We aimed to test these hypotheses in a population-based cohort. Between 2008 and 2014, we assessed genetic variants, cognitive functioning and gait in 4,987 participants of the Rotterdam Study who were free of parkinsonism and dementia (median age 68 years, 57% women). We constructed a weighted genetic risk score for PD based on 39 single nucleotide polymorphisms that were previously identified in genome-wide association studies. We used four cognitive tests to assess cognitive functioning, and calculated a Global Cognition score. We used an electronic walkway (GAITRite™) to assess gait and derived seven independent gait domains, and calculated a Global Gait score. Higher genetic risk was associated with worse Global Cognition overall (age- and sex-adjusted standardized β=0.03; p=0.01). After stratification by Global Gait, the association was only present in individuals with below-average gait (p for interaction term with Global Gait=0.01). The genetic risk score was not significantly associated with Global Gait overall (β=0.03; p=0.11), however, the association was modified by cognition (p for interaction term with Global Cognition<0.01) and was significant in individuals with below-average cognition. In conclusion, genetic variants implicated in PD are associated with cognitive functioning and gait in clinically unaffected individuals, possibly including prodromal PD patients. Genetic associations with cognitive performance are more distinct in individuals with below-average gait, and genetic

associations with gait are more distinct in individuals with below-average cognition.

BACKGROUND

Before patients can be diagnosed with clinical Parkinson's Disease (PD), they go through a phase in which prodromal signs such as subtle gait impairments and cognitive deficits gradually emerge.[1] Recent genome-wide association studies have identified 41 genetic variants implicated in PD,[2,3] however, it remains unclear to what extent these genetic variants affect the occurrence of PD features in individuals without clinical PD, including individuals who are in the prodromal phase of PD. Such insight would improve our understanding of phenotypic correlates of genetic variants implicated in PD.

In isolation, subtle cognitive deficits and subtle motor features (including subtle gait impairments) are each associated with a modestly increased risk of PD, and each can have various other underlying causes that are genetically unrelated to PD. However, the combination of subtle cognitive deficits and subtle motor features conveys a much higher risk of PD.[4]

We hypothesized that, in individuals who are free of parkinsonism and dementia, genetically elevated risk of PD is associated with worse cognitive functioning and gait. We also hypothesized that genetic associations with cognitive functioning would be more distinct in individuals with below-average gait, and, conversely, genetic associations with gait may be more distinct in those with below-average cognition. We aimed to improve our understanding of phenotypic correlates of genetic variants implicated in PD by testing these hypotheses in a large, population-based cohort with electronic gait assessments and an extensive cognitive test battery.

METHODS

Study population

The study was embedded in the Rotterdam Study (RS), a large, prospective, population-based study in the Netherlands.[5,6] In 1990, inhabitants of the well-defined Ommoord district in the city of Rotterdam who were aged 55 years and older were invited to participate, and 7,983 individuals agreed (first subcohort). In 2000, all inhabitants who had become 55 years of age and older or who moved into the study district since the start of the study were invited to be included in the Rotterdam Study, and 3011 agreed (second subcohort). The cohort was further extended in 2006 (third subcohort; age range 45 years and older) to a total of 14,926 participants (overall response 72%). Of these individuals, genotyping was successfully performed in 11,481 individuals.[6]

By 2014, the first subcohort had a total of up to five visits, whereas the second subcohort had four visits, and the third subcohort had two (mean interval between visits: four years). Gait assessments were implemented into the core protocol of the Rotterdam Study in 2009. 4,987 out of 6,832 (73%) surviving individuals with genetic data who were free of dementia or parkinsonism participated in the center visit round between 2009 and 2014. Of these 4,987 individuals, 4,793 had an extensive cognitive assessment, 3,472 had an electronic gait assessment, and 3,278 had both.

Genetic risk

Genotyping was performed using the Illumina 550K, 550K duo, and 610K quad arrays.[6] Samples were removed that had a call rate below 97.5%, gender mismatch, excess autosomal heterozygosity, duplicates or family relations and ancestry outliers, and variants were removed with call rate below 95.0%, failing missingness test, Hardy–Weinberg equilibrium p-value $<10^{-6}$, and minor allele frequency<1%. Genotypes were imputed using MACH/ minimac software[7] to the 1000 Genomes reference panel.

We studied 39 of the 41 single nucleotide polymorphisms identified in the most recent and largest genome-wide association studies (GWAS) of PD to date.[2,3] Two of these variants (rs17649553 and rs9275326) were not genotyped in our dataset, nor reliably imputed (R2<0.3), and also lacked a proxy variant, leaving 39 variants for analysis.

Since the increase in risk of PD is small for individual variants, we calculated a combined genetic risk score to enable detection of the collective associations. This risk score was constructed by adding up all the risk alleles per individual weighted by their log-transformed, reported effect size for the association with PD. A higher genetic risk score corresponds to more risk variants and thus a higher risk of PD.

Gait

Gait was evaluated using a 5.79-m long walkway (GAITRite™ Platinum; CIR systems, Sparta, NJ: 4.88-m active area; 120-Hz sampling rate). The reliability and validity of this device have been previously established.[8-11] The standardized gait

protocol comprises three walking conditions: normal, turning and tandem walk. In the normal walk, which was repeated up to eight times, participants walked at their usual pace across the walkway. We calculated mean values across these walks, apart from the first walk, which we considered a practice walk. In turning, participants walked at their usual pace, turned halfway, and returned to the starting position. In the tandem walk, participants walked heel-to-toe on a line across the walkway.

After visual inspection of all recordings, the walkway software calculated 30 parameters based on the recorded footfalls, including 25 from the normal walk, 2 from the turning walk and 3 from the tandem walk. In *Table 1*, we provide a description of these parameters.

Cognitive functioning

We previously published a detailed description of our assessment methods of cognitive functioning.[12] In short, we used the Stroop color word test,[13] Letter-Digit Substitution Test (LDST),[14] Word Fluency Test,[15] and the 15-Word List Learning Test (WLT).[16]

The abbreviated Stroop test consists of three subtasks in which the participant is shown a colored card with 40 items that have to be named.[13] In naming task, the participants are asked to name the printed words (primary latent domain: *speed of reading*); in the color task the participants are asked to name the printed colors (*speed of color naming*); in the interference task the participants are asked to name the color in which each color-name is printed (*information processing on an interference task*). For each trial, the time to complete the task was used as the

outcome; a higher score indicates a worse performance. The LDST is a modified version of the Symbol Digit Modalities Test[14] and asks the participants to make as many letter-digit combinations as possible in 60 seconds, following an example that shows correct combinations (*information processing speed / executive function*). In the Word Fluency Test, participants were asked to name as many animals as possible within 60 seconds (*semantic fluency*).[15] For both the Word Fluency Test and LDST the number of correct answers was used as the outcome. The WLT comprised of three tasks: immediate recall, delayed recall and recognition. For immediate recall, participants were presented three times with a sequence of 15 words and subsequently asked to recall as many of these words as possible (*verbal learning*). Free delayed recall was tested approximately 10 minutes later (*retrieval from verbal memory*). Recognition was tested by presenting the participants a sequence of 45 words, the 15 words presented during the Immediate recall mixed with 30 new words. Participants were asked whether they recognized the words as the ones presented to them during the immediate recall trial (*recognition from verbal memory*). Outcome variables were the mean of the number of words recalled over the first three trials (as a summary score for immediate recall), the number of words remembered after the 10-minute delay (as a score for free delayed recall) and the number of correctly recognized words during the recognition trial (as a score for recognition).

We note that we did not include the Purdue Pegboard Test for the current report, since it is strongly influenced by motor function.

Table 1 (part I) | Original gait parameters and correlating domains.

Parameter	*Description*	*"Worse" gait*	*Correlating domain*
Single Support Time	Time between the last contact of the opposite foot and the first contact of the next footfall of the opposite foot	higher	Rhythm
Swing Time	Time between the last contact of the current footfall to the first contact of the next footfall (same foot)	higher	Rhythm
Step Time	Time between the first contact of one foot and the first contact of the opposite foot	higher	Rhythm
Stride Time	Time between the first contacts of two consecutive footfalls (same foot)	higher	Rhythm
Cadence	Number of steps / minute	lower	Rhythm
Stance Time	Time between the first contact and the last contact of two consecutive footfalls of the same foot.	higher	Rhythm
Stride Length SD	Standard deviation in Stride Length	higher	Variability
Step Length SD	Standard deviation in Step Length	higher	Variability
Stride Velocity SD	Standard deviation in Stride Velocity	higher	Variability
Stride Time SD	Standard deviation in Stride Time	higher	Variability
Step Time SD	Standard deviation in Step Time	higher	Variability
Stance Time SD	Standard deviation in Stance Time	higher	Variability
Swing Time SD	Standard deviation in Swing Time	higher	Variability
Single Support Time SD	Standard deviation in Single Support Time	higher	Variability
Double Support Time SD	Standard deviation in Double Support Time	higher	Variability
Single Support (%GC)	Single Support Time as % of Stride Time	lower	Phases
Swing (%GC)	Swing Time as % of the Stride Time	lower	Phases
Stance (%GC)	Stance Time as % of the Stride Time	higher	Phases
Double Support (%GC)	Double Support Time as % of the Stride Time	higher	Phases

Table 1 (part II) | Original gait parameters and correlating domains.

Parameter	*Description*	*"Worse" gait*	*Correlating domain*
Double Support Time	Time that two feet are on the ground at the same time within one footfall	higher	Phases
Stride Length	Distance between the heel points of two consecutive footprints (same foot) on the line of progression	lower	Pace
Step Length	Distance between the heel points of two consecutive opposite footprints on the line of progression	lower	Pace
Velocity	Stride Length / stride time	lower	Pace
Sum of Feet Surface	Sum of the surfaces of the side steps* as % of the surface of a normal step	higher	Tandem
Sum of Step Distance	Sum of the distances of the side steps* from the line on the walkway	higher	Tandem
Double Step	Step with one foot followed by a step with the same foot, where both feet are on the line of the walkway	higher	Tandem
Turning Step Count	Number of steps within Turning Time	higher	Turning
Turning Time	Time between the last contact of the second foot before the turn and the first contact of the second foot after the turn.	higher	Turning
Stride Width SD	Standard deviation in Stride Width	higher	Base of support
Stride Width	Distance from heel center of one footprint to the line of progression formed by two footprints of the opposite foot	lower	Base of support

*SD = standard deviation, %GC = as a percentage of the stride time. *A sidestep was defined as a step next to the line on the walkway, which was followed by a step with the same foot or a step with the other foot.*

Statistical analysis

Gait parameters with a skewed distribution were log-transformed, and all continuous gait parameters were standardized into Z-scores. To summarize gait parameters into independent domains, we performed a principal component analysis (PCA) with Varimax rotation, as previously described in detail.[17] This yielded 7 gait domains with an eigenvalue > 1, which we labeled in accordance with the gait parameters that are highly correlated with that domain: Base of Support, Pace, Phases, Rhythm, Tandem, Turning and Variability.[17] Gait domains are illustrated in *Figure 1*. Global Gait was calculated by averaging the normal walk gait domains into a standardized Z-score.[17] Global Gait explained 87% of the variance in baseline gait parameter values.

We calculated Global Cognition as the first compound of an unrotated PCA that incorporates tasks from all available cognitive functioning tests. Although Stroop and WLT comprised several tasks, we only used data from the most complicated task of each (i.e., the interference task for Stroop and the 15-minute delayed recall task for WLT) in calculating the g-factor to prevent highly correlated tasks distorting factor loadings in the PCA. Baseline Global Cognition explained 54% of the variance in baseline cognitive test scores.

All 3,472 individuals who participated in gait assessments had analyzable data on the normal walk, but 198 (5%) individuals did not perform the tandem walk and 149 (4%) individuals did not perform the turning walk. We imputed missing data on the turning and tandem walk based on age, sex and normal walk gait parameters. Of all the 4,793 individuals who participated in the cognitive test battery, 717 (15%) study participants did not complete one or two cognitive functioning tasks that were used to calculate Global Cognition. We performed

multiple imputation using the mean of five imputations based on age, sex and other cognitive test scores. The distribution of gait and cognitive test scores before and after imputation was similar.

We used linear regression models to assess the association of the genetic risk score with Global Cognition and cognitive functioning test scores. We assessed whether there was effect modification by Global Gait of the association between the genetic risk score and Global Gait, by adding Global Cognition and the interaction term [genetic risk score*Global Cognition] to the model. We also assessed the association of the genetic risk score with Global Gait and independent gait domains, and separately added Global Cognition and the interaction term [genetic risk score*Global Cognition] to the model to assess effect modification by Global Cognition. In sensitivity analyses, we examined associations of single variants with cognition and gait. All analyses were adjusted for age and sex.

Data were handled and analyzed with the IBM SPSS Statistics version 23.0.0.0 (IBM Corp., Somers, NY) and R version 3.2.4. We adjusted the statistical significance threshold for multiple hypothesis testing of correlated variables.[18] For associations of single variants with Global Cognition and Global Gait the adjusted threshold was p=0.0013. For associations of single variants and cognitive tests the adjusted threshold was p=0.00016. For associations of single variants and independent gait domains the adjusted threshold was p=0.00018. Regression coefficients are presented per risk allele (i.e., the allele that corresponds with higher risk of PD[2,3]) or per standard deviation increase in genetic risk score.

Table 2 | Study population characteristics.

	Total population **[n=4,987]**	**Sample with cognitive data** **[n=4,793]**	**Sample with gait data** **[n= 3,472]**
Age, median [IQR]	68.0 [13.4]	68.1 [13.3]	66.7 [12.5]
Female gender, n [%]	2852 [57.2]	2736 [57.1]	1900 [54.7]
Mini-Mental State Exam, median [IQR]	28.0 [2.0]	28.0 [2.0]	29.0 [2.0]
Gait speed (cm/s), median [IQR]	123.1 [23.5]	123.1 [23.6]	123.1 [23.5]
Global Cognition, median [IQR]	0.1 [1.2]	0.1 [1.2]	0.3 [1.2]
Global Gait, median [IQR]	0.2 [1.2]	0.2 [1.2]	0.2 [1.2]

N, number of individuals. IQR, interquartile range. cm/s, centimeters per second.
For Global Cognition and Global Gait, higher values represent better performance.

RESULTS

Characteristics

Average age in the study population was 68 years and 57% of study participants were women.(*Table 2*) Median MMSE score was 28, while the median gait speed was 123cm/s.(*Table 2*) Population characteristics were similar in the sample with cognitive function data, while the sample with electronic gait data contained fewer women and had slightly higher MMSE and Global Cognition scores.

Genetic risk for PD and cognition

We observed 24 nominally significant associations between single variants and cognitive test scores, but none of these associations survived the multiple-hypothesis testing threshold. The strongest association (i.e., lowest p-value) we observed was of rs356182 with the immediate recall task of the Word learning

test (β=-0.06, p=0.0008). Seven SNPs were nominally associated with Global Cognition, of which the association of rs356182 had the lowest p-value (β=-0.05, p=0.008), but none of these associations survived multiple-hypothesis testing. The genetic risk score for PD was nominally associated with the Letter-digit substitution test, Stroop naming task, Stroop color task, Stroop interference task and immediate recall task of the Word learning test.(*Table 3*) The genetic risk score was also associated with Global Gait.(*Table 3*) The effect size of the association of the genetic risk score with Global Cognition was similar in individuals who also had data on gait (β=0.03; p=0.028) as in the total sample with data on cognition. Additional adjustment for Global Gait only marginally diluted the association (β=0.03; p=0.056).

We observed statistically significant effect modification by Global Gait of the association between the genetic risk score and Global Cognition (p for interaction term=0.014). As shown in *Figure 2A*, the strength of the association between the genetic risk score and Global Cognition increased linearly for decreasing values of Global Gait. In individuals with below-average gait, the genetic risk score was associated with the Word fluency task and immediate recall task of the Word learning test, and also with Global Cognition.(*Table 3*) In individuals with above-average gait, we observed no statistically significant associations of the genetic risk score with cognitive test scores or with Global Cognition.(*Table 3*)

Table 3 | Genetic risk score and cognition.

Cognitive test	Median score [IQR]	β of genetic risk score [p-value]		
		Total sample	Below-average gait*	Above-average gait**
Letter-digit substitution	29.0 [10.0]	-0.02 [0.048]	-0.06 [0.008]	0.00 [0.991]
Stroop naming	17.0 [3.9]	-0.03 [0.026]	-0.05 [0.062]	-0.03 [0.072]
Stroop color	23.4 [5.6]	-0.03 [0.042]	-0.05 [0.063]	-0.02 [0.368]
Stroop interference	47.4 [19.7]	-0.02 [0.071]	-0.04 [0.212]	-0.00 [0.803]
Word fluency	22.0 [7.0]	-0.02 [0.240]	-0.07 [0.004]	0.01 [0.504]
Word learning - immediate recall	8.0 [3.0]	-0.02 [0.167]	-0.03 [0.172]	-0.01 [0.593]
Word learning - delayed recall	8.0 [4.0]	-0.02 [0.071]	-0.07 [0.005]	0.00 [0.852]
Word learning - recognition	14.0 [3.0]	-0.02 [0.216]	0.00 [0.886]	-0.03 [0.131]
Global Cognition	***0.2 [1.2]***	***-0.03 [0.014]***	***-0.08 [<0.001]***	***0.00 [0.885]***

*β, age- and sex-adjusted standardized regression coefficient of genetic risk score for each standard deviation increase in cognitive or Global Cognition score. Higher cognitive test scores indicate better cognition (Stroop scores were multiplied by -1).*Global Gait z-score <0. **Global Gait z-score >0. Color indicates p-value of the association:*

≥0.05	<0.05	<0.01	<0.005

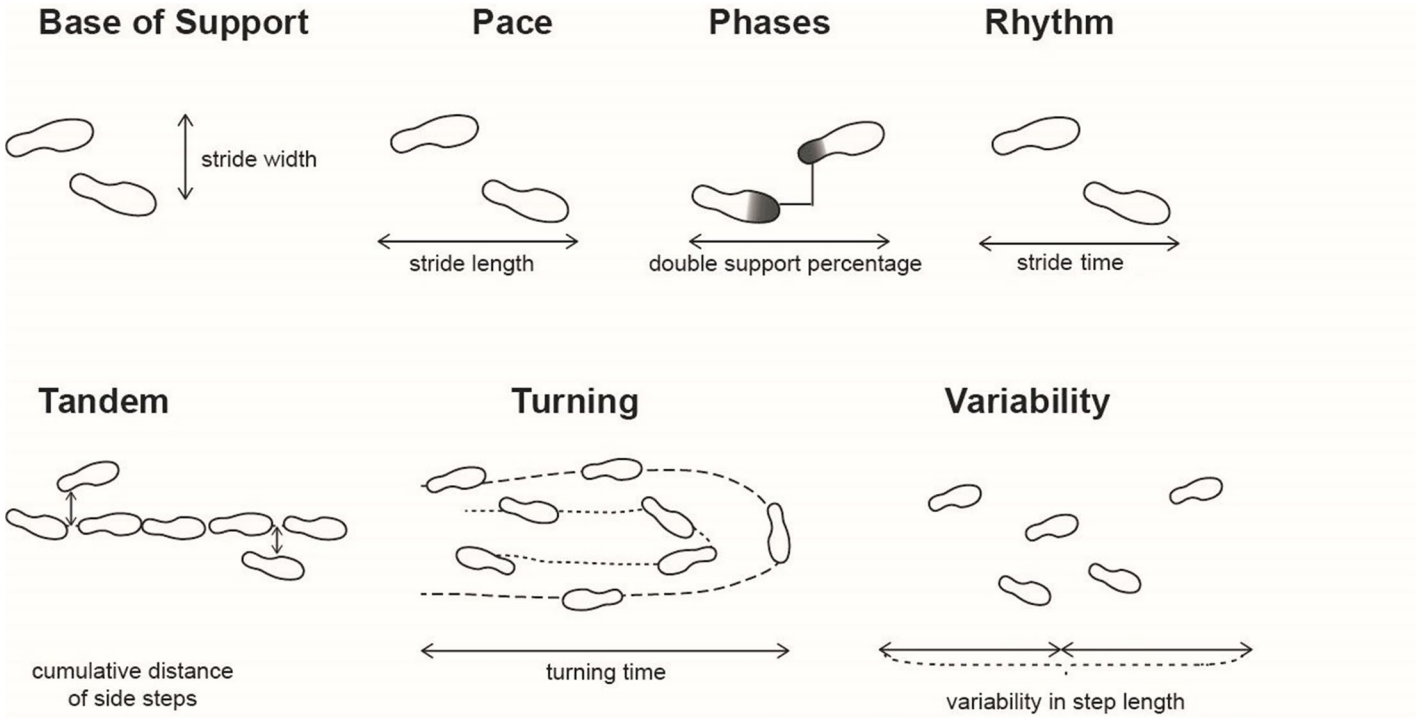
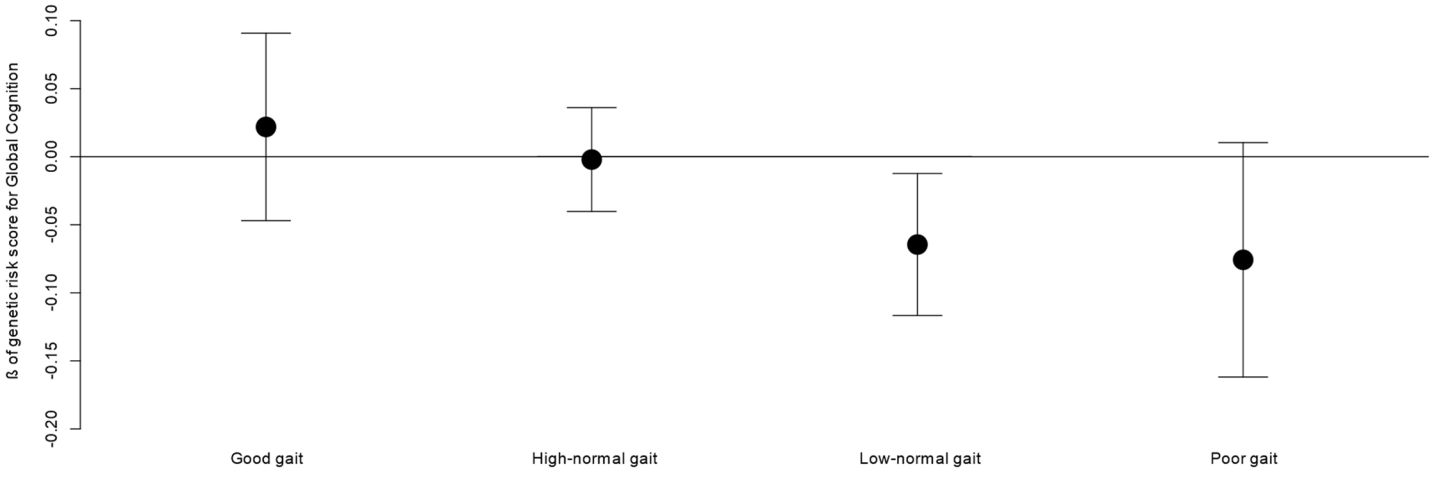


Figure 1 | Gait domains

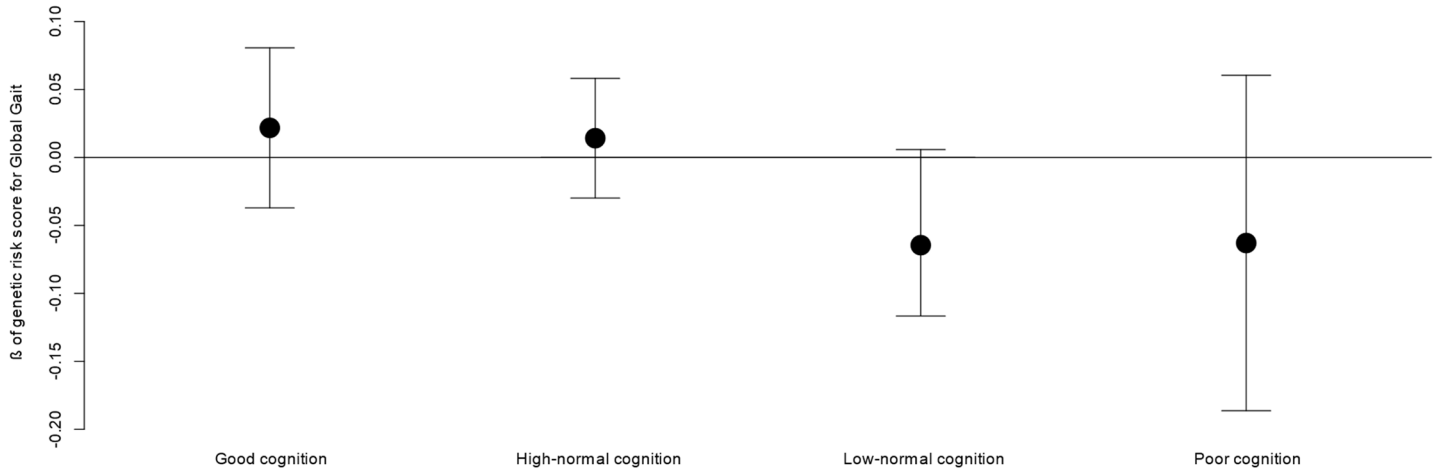
To summarize gait parameters into independent domains, we performed a principal component analysis. This yielded 7 independent gait domains: Base of Support, Pace, Phases, Rhythm, Tandem, Turning and Variability. For each gait domain, a single gait parameter that has high correlation with the domain is illustrated.

Figure 2 (part A) | Genetic risk of Parkinson's Disease, Global Cognition and Global Gait



Good gait, Global Gait z-score > 1. High-normal gait, Global Gait z-score [0 to 1]. Low-normal gait, Global Gait z-score [-1 to 0]. Poor gait, Global Gait z-score < -1. β, age- and sex-adjusted standardized regression coefficient of genetic risk score for each standard deviation increase in Global Cognition, bars indicate 95% confidence intervals.

Figure 2 (part B) | Genetic risk of Parkinson's Disease, Global Cognition and Global Gait



Good cognition, Global Cognition z-score > 1. High-normal cognition, Global Cognition z-score [0 to 1]. Low-normal cognition, Global Cognition z-score [-1 to 0]. Poor cognition, Global Cognition z-score < -1. β, age- and sex-adjusted standardized regression coefficient of genetic risk score for each standard deviation increase in Global Gait, bars indicate 95% confidence intervals.

Table 4 | Genetic risk score and gait.

Gait domain	β of genetic risk score [p-value]		
	Total sample	**Below-average cognition***	**Above-average cognition****
Base of Support	0.00 [0.845]	-0.02 [0.474]	0.03 [0.204]
Pace	-0.02 [0.219]	-0.01 [0.623]	-0.02 [0.356]
Phases	-0.02 [0.159]	-0.04 [0.171]	0.00 [0.984]
Rhythm	0.00 [0.968]	-0.01 [0.838]	0.01 [0.738]
Tandem	0.01 [0.520]	-0.03 [0.345]	0.04 [0.040]
Turning	0.00 [0.831]	-0.02 [0.416]	0.02 [0.292]
Variability	-0.04 [0.009]	-0.03 [0.205]	-0.04 [0.054]
Global Gait	-0.03 [0.105]	-0.06 [0.030]	0.02 [0.397]

*β, age- and sex-adjusted standardized regression coefficient of genetic risk score for each standard deviation increase in gait domain or Global Gait score. Higher gait domain values indicate better gait. *Global Cognition z-score <0. **Global Cognition z-score ≥0. Color indicates p-value of the association:*

≥0.05	<0.05	<0.01	<0.005

DISCUSSION

Genetic variants implicated in PD are associated with several cognitive tasks as well as high variability in gait in individuals free of parkinsonism and dementia. Interestingly, genetic associations with cognitive functioning are only present in those with below-average gait, and genetic associations with gait are most

apparent in those with below-average cognitive functioning. Our data suggest that genetic variants implicated in PD contribute to the occurrence of motor signs and cognitive dysfunction in individuals who are (still) free of clinical PD.

Before further interpreting the results, we note three limitations of this study. First, 30% of the study population did not undergo an electronic gait assessment, compared to only 4% who did not undergo a cognitive functioning assessment. As a consequence we had more statistical power to detect similar effect sizes of genetic associations with cognitive functioning than with gait. This may explain why the regression coefficients of the genetic risk score for Global Cognition and Global Gait were identical (β=0.03), but only the former was statistically significant. Second, our cognitive test battery was not designed specifically to detect cognitive dysfunction in PD[19] and our gait assessment lacked information on some specific PD traits such as difficulty initiating and terminating gait,[20] suggesting that genetic studies incorporating even more refined outcomes may uncover additional PD-associated genetic associations with cognition and gait. Third, we only assessed gait under single-task conditions, and genetic associations with gait may be amplified if gait is assessed under dual-task conditions (e.g., by asking participants to simultaneously perform a cognitive task).[21]

Subtle motor features and subtle cognitive deficits are common in the general population and can have various underlying causes that are genetically unrelated to PD. For instance, isolated slow gait can result from locomotor diseases, while isolated impaired memory recall can stem from cerebral small-vessel disease. As a consequence, subtle motor features and subtle cognitive deficits each convey a relatively modest increase in the risk of PD when they occur in isolation.[4,22] The combination of subtle motor features and subtle cognitive deficits, however,

conveys a substantially higher risk of PD.[4] Therefore, we hypothesized that genetic effects of PD-associated variants on gait would be more distinct in individuals with worse cognition, and vice versa. In line with our hypotheses, we observed that the strength of the association between the genetic risk score and cognitive functioning increased linearly as gait performance diminished, and that the association between the genetic risk score and gait became stronger as cognitive functioning declined. This suggests that genetic variants implicated in PD may influence cognition in prodromal PD patients, in individuals who never progress to clinical PD, or in both.

We observed that genetic variants implicated in PD are robustly associated with global cognitive functioning in individuals with below-average gait but not in individuals with above-average gait. Our data also provide some hints on possible genetic effects on specific cognitive tasks. We observed that genetic variants implicated in PD were associated with performance on several cognitive tasks that were previously implicated in prodromal probable PD[4,23]: information processing speed and executive function (assessed by letter-digit substitution test) and color naming (Stroop color task) in the total sample, and also semantic fluency (word fluency test) in individuals with below-average gait. We also observed associations of the genetic risk score with two tasks that were not previously implicated in prodromal probable PD in our cohort[4]: Stroop naming task (speed of reading; in the total sample) and delayed recall task of the word learning test (retrieval from verbal memory; in the sample with below-average gait). Replication of task-specific associations in other cohorts is warranted to confirm that genetic variants implicated in PD affect information processing speed, executive function, color naming, and semantic fluency in individuals free of

parkinsonism and dementia, and to explain the unexpected genetic association with speed of reading and retrieval from verbal memory.

Similar to the observed genetic associations with cognition, we observed that genetic variants implicated in PD are associated with Global Gait in individuals with below-average cognition but not in individuals with above-average cognition. We also assessed genetic associations with specific gait domains, because PD patients have a tendency for high step-to-step variability (Variability), time in double support (Phases), and several other quantitative gait impairments.[20,24] We observed an association between genetic variants implicated in PD with Variability, suggesting that that these genetic variants may affect step-to-step variability in individuals free of parkinsonism and dementia. In individuals with below-average cognitive functioning, no genetic associations with specific gait domains were statistically significant, although we note that the association with Phases had the highest non-significant regression coefficient. In individuals with below-average cognitive functioning, we observed an unexpected association of higher genetic risk for PD with better performance on a heel-to-toe walk (Tandem). Future studies that use even more refined gait phenotypes and implement dual-task walks are warranted to confirm these domain-specific genetic associations and to unravel additional possible genetic effects on gait.

In conclusion, genetic variants implicated in PD also affect cognitive functioning in individuals without parkinsonism or dementia, specifically in those with below-average gait. Interestingly, we also observed associations of these genetic variants with gait in individuals with below-average cognitive functioning. Leveraging simultaneous cognitive and gait assessments, we have uncovered associations of genetic variants implicated in PD with cognition and gait in clinically unaffected individuals, possibly including prodromal PD patients.

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Chapter 2.2

Cerebral microstructure

2

Chapter 2.2

ABSTRACT

We hypothesized that microstructural volumes across gray and white matter and subcortical shapes are associated with motor function performance in individuals free of clinical neurodegenerative disease. In 3,305 stroke-free individuals from the Rotterdam Study (mean age 67 years, 54% women) with brain-MRI and motor function assessments, we studied (sub)cortical gray matter and white matter in relation to upper and lower extremity motor function performance. We assessed gray matter using voxel-based morphometry, and white matter using diffusion-MRI parameters (fractional anisotropy and mean diffusivity) in 14 white matter tracts that we obtained through probabilistic tractography. We assessed 47 quantitative motor function parameters using the Archimedes Spiral Test, Purdue Pegboard Test and GAITRite™. We performed a principal component analysis with Varimax rotation on these parameters, rendering 11 independent motor function domains. We used age- and sex-adjusted linear regression models to assess associations of motor function domains with microstructural (sub)cortical gray matter and white matter. We observed a pattern of associations between impairment in white matter connections involved in pyramidal, extrapyramidal, visual, somatosensory, and proprioceptive systems and several motor functions. We also observed associations between several clusters of gray matter voxels with worse performance on the Gait Pace and Gait Phases domains. Interestingly, both volume and shape of the caudate nucleus and the angular gyrus were among the regions most distinctly associated with gait, and microstructural impairment in the tract that connects these regions (inferior fronto-occipital fasciculus) was also associated with Gait Phases. These results suggest that microstructural barin deficits contribute to variability in motor function performance among individuals free of clinical neurodegenerative disease.

BACKGROUND

Impairments in upper and lower extremity motor functions are a hallmark of neurodegenerative diseases such as Alzheimer's Disease, Parkinson's Disease (PD) or frontotemporal dementia. In individuals with these diseases, motor impairments are often caused by underlying macrostructural brain deficits. Intriguingly, there is also substantial variability in motor functioning among individuals who lack macrostructural brain deficits, however, the underlying biological mechanisms accounting for this variability remain largely elusive. This is a critical gap in knowledge, since it contributes to the erroneous notion that impairment in motor functioning is an inevitable consequence of advancing age, possibly delaying clinical care seeking and precluding optimal secondary prevention of further motor decline.

The recent advance of imaging modalities has made it possible to assess cerebral microstructure in live humans. This has led to the revelation that cerebral microstructural deficits contribute to the variability in motor functioning in individuals with neurodegenerative diseases.[1-3] By contrast, empirical data on cerebral microstructural determinants of motor functioning in individuals who lack a clear underlying macrostructural brain deficit are relatively scarce. In particular, while we previously demonstrated associations of several white matter microstructure tracts with gait and manual dexterity,[4,5] the role of (sub)cortical gray matter on motor functioning remains unknown. Furthermore, it is largely unclear how microstructural gray matter deficits involved in motor functioning relate to microstructural white matter deficits.

We hypothesized that microstructural deficits contribute to variability in motor function performance among individuals who lack a clear underlying

macrostructural brain deficit. We also hypothesized that overlapping microstructural deficits across gray and white matter converge into worse motor functioning. To test these hypotheses, we assessed cerebral microstructure and upper and lower extremity motor functions in a general population.

METHODS

Study population

The study was embedded in the Rotterdam Study (RS), a large, prospective, population-based study in the Netherlands.[6,7] In 1990, inhabitants of the well-defined Ommoord district in the city of Rotterdam who were aged 55 years and older were invited to participate, and 7,983 individuals agreed (first subcohort). In 2000, all inhabitants who had become 55 years of age and older or who moved into the study district since the start of the study were invited to be included in the Rotterdam Study, and 3,011 agreed (second subcohort). The cohort was further extended in 2006 (third subcohort; age range 45 years and older) to a total of 14,926 participants (overall response 72.0%). By 2014, the first subcohort had a total of six visits, whereas the second subcohort had four visits, and the third subcohort had two (mean interval between visits: four years).

Brain MRI assessments were implemented in the core protocol of the Rotterdam Study in 2005, and have been conducted at all subsequent center visits. Motor function was assessed using three modalities: Purdue Pegboard test, Archimedes Spiral Test and GAITrite. In total, these modalities yielded 47 motor function parameters.(*Table 1*) The Purdue Pegboard test was introduced in 2000 and has been conducted at all subsequent visits. The Archimedes Spiral Test was assessed

at visits between 2008 and 2014, and gait assessments (GAITRite) at all visits from 2009 onwards. For the current project, we used data from the 2009-2014 period in which all three motor function modalities were assessed. During that period, 4,025 individuals underwent a gait assessment. 389 of these individuals did not undergo an Archimedes Spiral Test and, of the remaining individuals, 307 did not undergo a Purdue Pegboard test assessment and a further 24 did not undergo an MRI assessment, leaving 3,305 in the analyses.

Brain MRI

Brain MRI was performed on a 1.5-T MRI scanner (Signa Excite II, General Electric Healthcare, Milwaukee, WI,USA) using an eight-channel head coil. The protocol included T1-weighted sequence (T1), proton density-weighted sequence, and a T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence, as described extensively in detail before[8].

Voxel based morphometry (VBM) was performed according to an optimized VBM protocol[9] and as previously described.[10] Briefly, all T1-weighted images were segmented into supratentorial gray matter, white matter and cerebrospinal fluid using a previously described k-nearest neighbor algorithm, which was trained on six manually labeled atlases[11]. All gray matter (GM) density maps were non-linearly registered to the standard ICBM MNI152 gray matter template (Montreal Neurological Institute) with a 1x1x1 mm^3 voxel resolution. A spatial modulation procedure was used to avoid differences in absolute gray matter volume due to the registration, following by smoothing procedure, using a 3mm (FWHM 8mm) isotropic Gaussian kernel.

Subcortical shapes

The T1-weighted MRI scans were processed using FreeSurfer[12] (version 5.1) to obtain segmentations and volumetric summaries of the following seven subcortical structures for each hemisphere: nucleus accumbens, amygdala, caudate, hippocampus, pallidum, putamen, and thalamus.[13] Next, segmentations were processed using a previously described shape analysis pipeline.[13,14] Briefly, a mesh model was created for the boundary of each structure. Subcortical shapes were registered using the "Medial Demons" framework, which matches shape curvatures and medial features to a pre-computed template.[15] The templates and mean medial curves were previously constructed and are distributed as part of the ENIGMA-Shape package (http://enigma.usc.edu/ongoing/enigma-shape-analysis/). The resulting meshes for the 14 structures consist of a total of 27,120 vertices.[13] Two measures were used to quantify shape: the radial distance and the natural logarithm of the Jacobian determinant. The radial distance represents the distance of the vertex from the medial curve of the structure. The Jacobian determinant captures the deformation required to map the subject-specific vertex to a template and indicates shape dilation due to sub-regional volume change.[13]

Purdue Pegboard test

In the Purdue Pegboard Test, participants are asked to place as many cylindrical metal pegs into 1 of 25 holes in a pegboard as possible in 30 seconds. The test is performed three times (left hand, right hand and both hands simultaneously). We used test scores on each separate task, the mean Purdue Pegboard Test score across tasks, and the difference between tasks executing using left vs. right arm.

Archimedes Spiral Test

In the Archimedes Spiral Test, participants are tasked to trace a template of a spiral on an electronic drawing board. All electronic recordings were visually inspected, and we have previously reported processing methods in detail.[17] From each drawing, we automatically calculated: path length, movement time, average absolute radial distance between template and drawing, number of direction changes and number of crossings (of template). In addition, we calculated the following derivatives of these parameters: average drawing speed, area of drawing deviation, standard deviation of absolute radial distance, standard deviation of drawing speed, density of direction changes, density of crossings.

GAITRite

Gait was evaluated using a 5.79-m long walkway (GAITRite Platinum; CIR systems, Sparta, NJ: 4.88-m active area; 120-Hz sampling rate). The reliability and validity of this device have been previously established.[18-21] The standardized gait protocol comprises three walking conditions: normal, turning and tandem walk. In the normal walk, which was repeated up to eight times, participants walked at their usual pace across the walkway. We calculated mean values across these walks, apart from the first walk, which we considered a practice walk. In turning, participants walked at their usual pace, turned halfway, and returned to the starting position. In the tandem walk, participants walked heel-to-toe on a line across the walkway. Based on the recorded footfalls, the walkway software

calculated thirty 30 parameters, including 25 from the normal walk, two from turning and three from the tandem walk. All recordings were visually inspected.

Statistical analysis

We performed a principal component analysis with Varimax rotation on all 47 motor function parameters, and labelled independent domains with an eigenvalue > 1 in accordance with the motor function parameters that were highly correlated with that domain. This yielded 11 independent domains with an eigenvalue > 1: Base of Support, Hypermetria, Manual Dexterity, Manual Precision, Pace, Phases, Rhythm, Tandem, Tremor, Turning and Variability.(*Table 1*, *Figure 1*) We standardized all motor function parameters and domains.

For both gray and white matter microstructure analyses, we used linear regression models with age, sex and motor function parameter value as independent variables and voxel measure as the dependent variable. For white matter microstructure analyses, we used standardized FA and standardized MD as outcomes in separate analyses. We computed the significance threshold based on nonparametric statistic test by performing 10,000 random permutations.[22] After collecting the minimum p-value from every test, they were sorted and the 5% quantile was used (α=0.05) to estimate the p-value significant threshold, while controlling the family wise error (FWE). We further divided the FWE-threshold by the number of independent motor function domains (n=11) to account for multiple hypothesis tests, setting the overall adjusted threshold to $p=2.7*10^{-8}$ for VBM analyses and $8.8x10^{-7}$ for shape analyses. We mapped gray matter microstructure results onto brain regions, and mapped white matter microstructure results onto white matter tracts.

Table 1 (part I) | Motor function parameters and latent motor function domains.

Extremities	Activity	Motor function parameter	Description	Worse motor performance
Upper body	Drawing of spiral	Movement Time	Movement time (seconds)	Higher
		Drawing speed	Average drawing speed (cm/s)	Lower
		Average absolute radial distance	Average absolute radial distance between template and spiral (cm)	Higher
		Area of drawing deviation	Cumulative area of deviation from template (cm2) [= Path Length * Average absolute radial distance]	Higher
		Standard deviation of absolute radial distance	Standard deviation of absolute radial distance between template and spiral (cm)	Higher
		Standard deviation of Drawing speed	Standard deviation of drawing speed (cm/s)	Higher
		Path length	Path length of spiral (cm)	Higher
		Direction changes	Number of movements in opposite direction	Higher
		Density of direction changes	Number of movements in opposite direction divided by path length	Higher
		Crossings	Number of times the spiral crossed the template	Higher
		Density of crossings	Number of times the spiral crossed template divided by path length	Higher
	Placing pins pegboard	Purdue left	Purdue Pegboard score (left-hand task)	
		Purdue right	Purdue Pegboard score (right-hand task)	
		Purdue both	Purdue Pegboard score (biupper extremity tasks)	Lower
		Purdue sum	Purdue Pegboard score (sumscore of left, right and bimanual tasks)	
		Purdue asymmetry	Difference in Purdue Pegboard score (left- vs. right-hand task)	

Latent domain	
Manual Precision	
Hyper-metria	
Tr.	
Manual dexterity	

Table 1 (part II) | Motor function parameters and latent motor function domains.

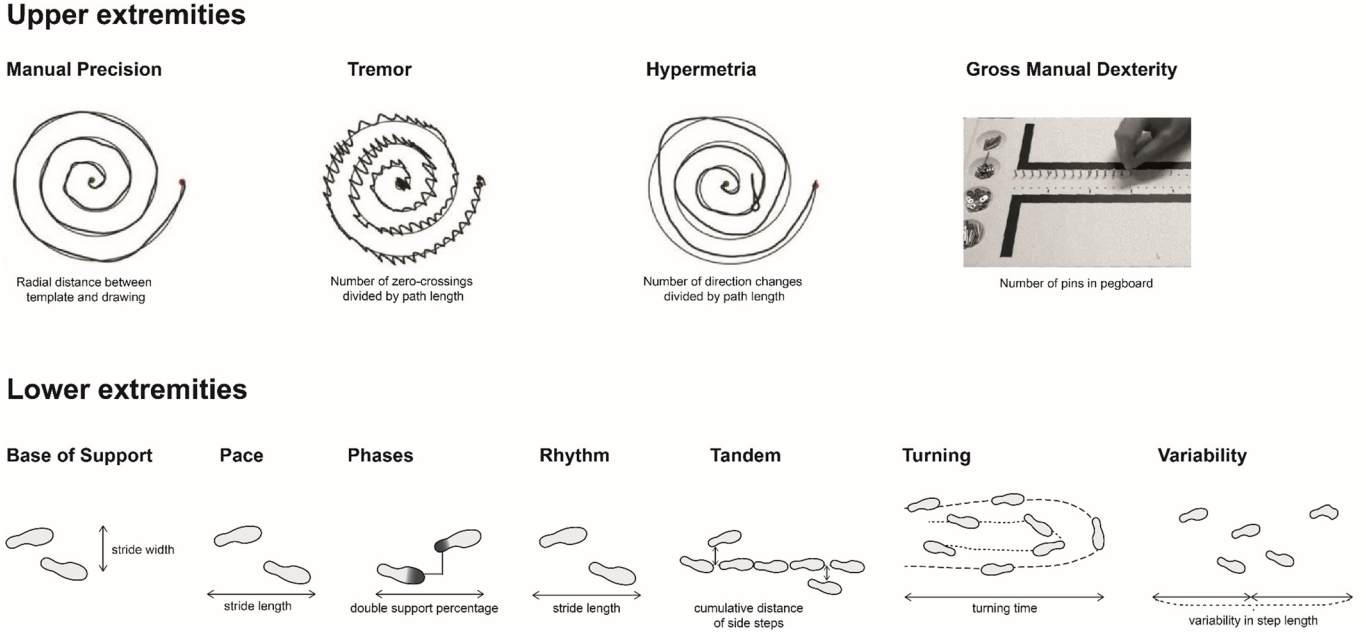
Extremities	Activity	Latent domain	Motor function parameter	Description	Worse motor performance
Lower body	Normal walk	Gait Rhythm	Single Support Time	Time between the last contact of the opposite foot and the first contact of the next footfall of the opposite foot	Higher
			Swing Time	Time between the last contact of the current footfall to the first contact of the next footfall (same foot)	Higher
			Step Time	Time between the first contact of one foot and the first contact of the opposite foot	Higher
			Stride Time	Time between the first contacts of two consecutive same footfalls	Higher
			Cadence	Number of steps / minute	Lower
			Stance Time	Time between the first contact and the last contact of two consecutive footfalls of the same foot.	Higher
		Gait Variability	Stride Length SD	Standard deviation in Stride Length	Higher
			Step Length SD	Standard deviation in Step Length	Higher
			Stride Velocity SD	Standard deviation in Stride Velocity	Higher
			Stride Time SD	Standard deviation in Stride Time	Higher
			Step Time SD	Standard deviation in Step Time	Higher
			Stance Time SD	Standard deviation in Stance Time	Higher
			Swing Time SD	Standard deviation in Swing Time	Higher
			Single Support Time SD	Standard deviation in Single Support Time	Higher
			Double Support Time SD	Standard deviation in Double Support Time	Higher
		Gait Phases	Single Support (%GC)	Single Support Time as % of Stride Time	Lower
			Swing (%GC)	Swing Time as % of the Stride Time	Lower
			Stance (%GC)	Stance Time as % of the Stride Time	Higher
			Double Support (%GC)	Double Support Time as % of the Stride Time	Higher
			Double Support Time	Time that two feet are on the ground at the same time	Higher

Table 1 (part III) | Motor function parameters and latent motor function domains.

Extremities	Activity	Latent domain	Motor function parameter	Description	Worse motor perfor-mance
Lower body	Normal walk	Gait Pace	Stride Length	Distance between the heel points of two consecutive footprints (same foot) on the line of progression	Lower
			Step Length	Distance between the heel points of two consecutive opposite footprints on the line of progression	Lower
			Velocity	Stride Length / stride time	Lower
		Gait Base of Supp.	Stride Width SD	Standard deviation in Stride Width	Higher
			Stride Width	Distance from heel center of one footprint to the line of progression formed by two footprints of the opposite foot	Lower
	Heel-to-toe walk	Gait Tandem	Sum of Feet Surface	Sum of the surfaces of the side steps* as % of the surface of a normal step	Higher
			Sum of Side Step Distance	Sum of the distances of the side steps* from the line on the walkway	Higher
			Double Step	Step with one foot followed by a step with the same foot, where both feet are on the line of the walkway	Higher
	Turning walk	Gait Turning	Turning Step Count	Number of steps within Turning Time	Higher
			Turning Time	Time between the last contact of the second foot before the turn and the first contact of the second foot after the turn.	Higher

*SD = standard deviation, Tr. = Tremor, Supp. = Support, %GC = as a percentage of the stride time. *A sidestep was defined as a step next to the line on the walkway, which was followed by a step with the same foot or a step with the other foot.*

Figure 1 | Motor domains.



RESULTS

Characteristics

Population characteristics are presented in *Table 2*. The average age in the study population was 67 years and 54% of study participants were women.

Table 2 | Study population characteristics.

	Total population n=3,305
Age, years	66.9 (8.1)
Female	1,781 (53.9)
Education, years	12.7 (3.9)
Right handedness, %	2,912 (88.1)
Gait speed, cm/s	123.4 (18.3)
Left hemisphere, cm^3	
Nucleus accumbens	0.55 (0.09)
Amygdala	1.30 (0.21)
Caudate nucleus	3.37 (0.55)
Hippocampus	3.82 (0.63)
Pallidum	1.46 (0.23)
Putamen	4.56 (0.63)
Thalamus	6.15 (0.73)
Right hemisphere, cm^3	
Nucleus accumbens	0.48 (0.09)
Amygdala	1.39 (0.22)
Caudate nucleus	3.48 (0.56)
Hippocampus	3.84 (0.59)
Pallidum	1.39 (0.24)
Putamen	4.40 (0.61)
Thalamus	6.16 (0.73)

Data presented as mean (standard deviation) for continuous variables and number (percentages) for categorical variables.

The correlation between motor function parameters varied between (inverted) Pearson correlation ρ 0.00-0.99. the correlation of motor function parameters with independent domains varied between (inverted) ρ 0.00-0.98.(*Table 3)* Of Archimedes Spiral test parameters, we observed very high ([inverted] $\rho \geq 0.75$) correlations between drawing speed, standard deviation of drawing speed, and movement time, and moderate ([inverted] $\rho \geq 0.25$) to high ([inverted] $\rho \geq 0.50$) correlations of these parameters with path length, area of drawing deviation, average absolute radial distance, and standard deviation of absolute radial distance. These parameters correlated moderately to very highly with the Manual Precision domain. We also observed very high correlations between number of crossings and crossing density and moderate correlations of both parameters with path length. These parameters correlated moderately to very highly with the Tremor domain. Similarly, the number of direction changes and direction change density highly intercorrelated and both moderately correlated with path length, and these parameters correlated moderately to very highly with the Hypermetria domain.

Motor function parameters

Purdue Pegboard test parameters very highly intercorrelated and correlated very highly with the Manual Dexterity domain, except the left-right difference in Purdue score, which lowly ($\rho < 0.10$) moderately correlated with the other Purdue Pegboard test parameters and moderately correlated with the Manual Dexterity domain. Tandem walk parameters (Double Step, Sum of Step Distance, Sum of Feet Surface) correlated mildly ($\rho \geq 0.10$) to very highly, and each correlated highly or very highly with the Tandem domain.

Table 3 (part I) | Correlation between original motor function parameters and domains.

Description	Rhythm	Phases	Variability	Manual precision	Manual dexterity	Pace	Hyper-metria	Tremor	Tandem	Turning	Base of Support
Average absolute radial distance	-0.01	0.05	0.04	0.61	0.08	-0.05	0.13	-0.36	0.01	0.03	0.38
Drawing speed	0.03	-0.14	0.02	0.85	0.01	0.10	-0.15	0.29	0.01	-0.07	-0.22
Area of drawing deviation	0.00	0.10	0.06	0.78	0.11	-0.05	0.15	-0.32	0.00	0.05	0.33
Density of crossings	0.02	-0.04	0.13	0.04	0.12	0.08	0.11	0.93	0.01	-0.02	0.04
Density of direction changes	-0.02	0.01	0.06	0.00	0.04	0.09	0.96	0.06	0.05	0.01	-0.04
Movement Time	-0.02	0.09	0.02	-0.89	0.04	-0.04	0.23	-0.17	0.00	0.03	0.21
Direction changes	-0.02	0.01	0.06	0.00	0.04	0.09	0.96	0.07	0.05	0.01	-0.03
Crossings	0.02	-0.04	0.13	0.02	0.12	0.08	0.16	0.92	0.01	-0.02	0.05
Standard deviation of absolute radial distance	0.00	0.06	0.05	0.84	0.14	-0.01	0.10	-0.16	0.02	0.03	0.20
Standard deviation of Drawing speed	0.01	-0.07	0.02	0.89	0.00	0.06	-0.07	0.21	0.02	-0.02	-0.19
Path length	0.03	0.01	0.05	-0.43	0.02	0.05	0.57	0.34	-0.02	0.00	0.09
Purdue both	-0.11	-0.01	-0.13	-0.06	-0.85	-0.06	-0.03	-0.07	-0.08	-0.06	0.01
Purdue difference left-right	-0.04	0.02	-0.02	-0.02	0.33	0.01	-0.03	-0.05	-0.03	-0.02	0.04
Purdue left	-0.09	-0.02	-0.12	-0.06	-0.86	-0.06	-0.04	-0.09	-0.04	-0.02	0.04
Purdue right	-0.13	-0.03	-0.13	-0.06	-0.81	-0.07	-0.06	-0.12	-0.06	-0.03	0.06
Purdue sum	-0.12	-0.02	-0.14	-0.07	-0.96	-0.07	-0.05	-0.11	-0.07	-0.04	0.04
Stride Width	0.10	0.10	0.27	0.07	0.14	-0.12	0.06	-0.07	0.01	0.07	-0.67
Cadence	-0.93	-0.29	-0.14	0.00	-0.07	-0.03	0.01	0.00	0.02	-0.05	0.02
Stride Time	0.92	0.33	0.14	0.00	0.07	0.05	-0.01	0.01	-0.02	0.05	-0.03
Double Support Time	0.44	0.86	0.08	-0.01	0.04	0.12	0.00	-0.01	0.02	0.05	-0.03
Double Support (%GC)	0.05	0.98	0.03	-0.02	0.02	0.15	0.01	-0.02	0.04	0.05	-0.01
Single Support (%GC)	-0.05	-0.98	-0.03	0.03	-0.02	-0.13	-0.01	0.03	-0.04	-0.05	0.02
Single Support Time	0.94	-0.27	0.13	0.01	0.07	-0.03	-0.01	0.02	-0.04	0.03	-0.01

Table 3 (part I) | Correlation between original motor function parameters and domains.

Description	Rhythm	Phases	Variabi-lity	Manual precisio	Manual dexterit	Pace	Hyper-metria	Tremor	Tandem	Turning	Base of Support
Stance (%GC)	0.05	0.98	0.03	-0.03	0.02	0.13	0.01	-0.03	0.04	0.05	-0.02
Stance Time	0.79	0.57	0.12	0.00	0.07	0.08	0.00	0.00	-0.01	0.05	-0.03
Step Length	0.11	-0.26	-0.17	-0.04	-0.10	-0.87	-0.10	-0.08	-0.10	-0.13	-0.03
Step Time	0.92	0.33	0.14	0.00	0.07	0.05	-0.01	0.01	-0.02	0.05	-0.02
Stride Length	0.11	-0.26	-0.17	-0.04	-0.10	-0.87	-0.10	-0.08	-0.09	-0.13	-0.03
Swing (%GC)	-0.05	-0.98	-0.03	0.03	-0.02	-0.13	-0.01	0.03	-0.04	-0.05	0.02
Swing Time	0.94	-0.27	0.13	0.01	0.07	-0.03	-0.01	0.02	-0.04	0.03	-0.01
Double Support Time SD	0.31	0.15	0.57	0.03	0.05	0.38	-0.01	0.07	0.00	0.06	0.11
Single Support Time SD	0.40	-0.01	0.62	0.05	0.15	0.46	0.05	0.04	0.07	0.04	-0.11
Stance Time SD	0.35	0.14	0.75	0.03	0.06	0.37	0.02	0.05	0.02	0.02	0.04
Step Length SD	0.03	0.02	0.86	0.03	0.16	-0.10	0.07	0.08	0.06	0.05	-0.14
Step Time SD	0.33	0.15	0.73	0.02	0.07	0.38	0.02	0.03	0.04	0.01	0.00
Stride Length SD	0.02	0.01	0.87	0.01	0.13	-0.12	0.06	0.07	0.05	0.05	-0.13
Stride Time SD	0.32	0.14	0.75	0.01	0.04	0.36	0.01	0.03	0.03	0.02	0.06
Stride Velocity SD	-0.17	-0.11	0.88	-0.02	0.01	-0.06	0.00	0.05	0.00	-0.01	0.04
Stride Width SD	0.03	-0.05	0.27	0.06	0.15	-0.26	-0.01	0.19	0.21	0.05	0.37
Swing Time SD	0.40	-0.01	0.62	0.05	0.15	0.46	0.05	0.04	0.07	0.04	-0.11
Velocity	-0.41	-0.35	-0.21	-0.03	-0.12	-0.73	-0.08	-0.07	-0.07	-0.13	-0.01
Double Step	-0.03	0.12	0.03	-0.03	-0.02	-0.03	0.00	-0.04	0.51	0.00	-0.01
Sum of Step Distance	0.00	-0.05	0.05	0.04	0.09	0.12	0.03	0.04	0.91	0.03	0.03
Sum of Feet Surface	-0.02	-0.01	0.06	0.05	0.09	0.13	0.07	0.05	0.92	0.02	0.03
Turning Step Count	-0.06	0.09	0.07	0.00	0.04	0.11	0.01	-0.03	0.04	0.94	-0.04
Turning Time	0.29	0.12	0.08	-0.02	0.02	0.17	0.01	-0.02	0.00	0.87	0.00

SD = standard deviation, %GC = as a percentage of the stride time. Color indicates (inverted) Pearson correlation:

<0.25	≥0.25	≥0.50	≥0.75

Turning walk and Turning Step Count very highly intercorrelated and both parameters highly correlated with the Turning domain. Correlations between normal walk parameters varied substantially, and these parameters clustered within five domains: Base of Support, Pace, Phases, Rhythm, Tandem, Variability. Interestingly, Archimedes Spiral test parameters mildly correlated with some parameters assessed during the Purdue Pegboard test, normal walk and tandem walk, but not with turning walk parameters. Furthermore, Purdue Pegboard test parameters mildly correlated some tandem walk and turning walk parameters, and mildly to moderately with some normal walk parameters. Normal walk parameters also mildly correlated with some tandem walk parameters, and mildly to moderately correlated with some turning walk parameters. We observed virtually no correlations between tandem and turning walk parameters.

Microstructural volumes: grey matter

Small regions in the right Cingulate gyrus (posterior part) and Postcentral gyrus were each associated with Manual Dexterity.(*Table 4*) Small regions in the left Postcentral gyrus, Lateral ventricle frontal horn central part and occipital horn, and Insula were each inversely associated with Pace. The cuneus, Lingual gyrus, and Postcentral gyrus on both sides (including a very small [8 voxel] region on the left) on both sides, left Lateral remainder of occipital lobe, and right Postcentral gyrus and Thalamus were each inversely associated with Tremor. The Caudate nucleus, Cuneus, Insula, Lingual gyrus, Middle frontal gyrus, Superior frontal gyrus, and Postcentral gyrus (including a very small [1 voxel] region on the left) on both sides were each associated with Phases. The left Putamen, right Superior temporal gyrus (central part), a very small region (3 voxels) of the right Anterior temporal lobe medial part, and a very small region (1 voxel) of the right Superior

parietal gyrus were each associated with Phases. Interestingly, small regions (<50 voxels) of the Lateral remainder of occipital lobe on both sides were associated with Phases with opposing effect directions for the left (positive association) and right (inverse association) side.

Of brain regions that were associated with more than one motor domain, we observed that the right Postcentral gyrus was associated with both Phases and Manual Dexterity. We also observed several associations of brain regions that were opposite in direction for different motor domains: Cuneus and Lingual gyrus on both sides, left Lateral remainder of the occipital lobe, and right Superior parietal gyrus were each associated with Phases and inversely associated with Tremor. Also, the left insula and left Postcentral gyrus were each associated with Phases and inversely associated with Pace.

Microstructural volumes: white matter

Regions within the Anterior thalamic radiation, Corticospinal tract, Inferior fronto-occipital fasciculus, Inferior longitudinal fasciculus, Posterior thalamic radiation, Superior longitudinal fasciculus, and Superior thalamic radiation were each associated with Manual Dexterity and Pace.(*data not shown*) Furthermore, regions within the left Medial lemniscus, Cingulate gyrus part of the cingulum (both sides), Forceps major, and Fornix were each associated with Pace. Also, regions within the Forceps minor and right Medial lemniscus were associated with Manual Dexterity. Regions within the Uncinate fasciculus were associated with Manual Dexterity (left side only) and Pace (both sides). Regions within the right Cingulate gyrus part of the cingulum and Corticospinal tract were associated with Phases.

Table 4 | Microstructural grey matter determinants of motor functioning.

Brain region	Side	Region size (# voxels)	# Neg. voxels	# Pos. voxels	Domain
Anterior temporal lobe medial part	right	11699	0	3	Phases
Caudate nucleus	left	6059	0	357	Phases
Caudate nucleus	right	6170	0	866	Phases
Cingulate gyrus posterior part	right	12439	0	8	Manual Dexterity
Cuneus	left	14454	0	658	Phases
Cuneus	right	13755	0	20	Phases
Cuneus	left	14454	5164	0	Tremor
Cuneus	right	13755	457	0	Tremor
Insula	left	21930	47	0	Pace
Insula	left	21930	0	640	Phases
Insula	right	22398	0	961	Phases
Lateral remainder of occipital lobe	left	64895	0	42	Phases
Lateral remainder of occipital lobe	right	66957	17	0	Phases
Lateral remainder of occipital lobe	left	64895	10024	0	Tremor
Lateral ventricle frontal horn central part & occipital horn	left	11711	37	0	Pace
Lingual gyrus	left	18132	0	96	Phases
Lingual gyrus	right	18495	0	39	Phases
Lingual gyrus	left	18132	3351	0	Tremor
Lingual gyrus	right	18495	858	0	Tremor
Middle frontal gyrus	left	80119	0	115	Phases
Middle frontal gyrus	right	81880	0	125	Phases
Postcentral gyrus	right	42995	0	4	Manual Dexterity
Postcentral gyrus	left	46092	7	0	Pace
Postcentral gyrus	left	46092	0	1	Phases
Postcentral gyrus	right	42995	0	147	Phases
Postcentral gyrus	right	42995	29	0	Tremor
Putamen	left	7438	0	28	Phases
Superior frontal gyrus	left	82424	0	169	Phases
Superior frontal gyrus	right	84342	0	153	Phases
Superior parietal gyrus	right	67336	0	1	Phases
Superior parietal gyrus	left	63572	448	0	Tremor
Superior parietal gyrus	right	67336	8	0	Tremor
Superior temporal gyrus, central part	right	21840	0	69	Phases
Thalamus	right	10429	61	0	Tremor

All associations are adjusted for age and sex. Neg. = negative. Pos. = positive.

Subcortical shapes

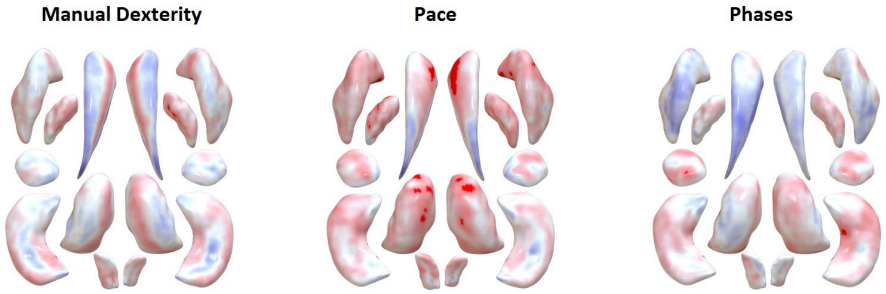
Associations between subcortical shapes and independent motor domains are listed in *Table 5* and illustrated in *Figure 2*. Thickness of the Thalamus on both sides was associated with Manual Dexterity, Pace, and Phases. Thickness of the Amygdala on both sides was associated with Pace and Phases. Furthermore, thickness of the Caudate, Putamen and Pallidum on both sides as well as thickness of the right Accumbens were each associated with Pace. Also, thickness of the left Pallidum was associated with the Manual Dexterity, and thickness of the left Hippocampus was associated with Phases. Aside from associations of thickness with motor domains, we also observed associations with Pace of the Jacobian determinant of the Pallidum (both sides), left Putamen, right Thalamus and right Accumbens. Subcortical shapes were not associated with other independent motor domains.

Table 5 | Subcortical shapes and independent motor domains.

Structure	Side	Manual Dexterity (#Neg. voxels)	Pace (#Neg. voxels)	Phases (#Neg. voxels)
Thalamus	Left	10	28	31
Thalamus	Right	1	83	17
Caudate	Left	0	100	0
Caudate	Right	0	24	0
Putamen	Left	0	70	0
Putamen	Right	0	17	0
Pallidum	Left	11	25	0
Pallidum	Right	0	88	0
Hippocampus	Left	0	0	10
Amygdala	Left	0	21	9
Amygdala	Right	0	33	2
Accumbens	Right	0	14	0

All associations are adjusted for age and sex. Neg. = negative. Pos. = positive.

Figure 2 | Subcortical shapes and motor domains.



Color map represents the t-statistics and shows the direction of association, with blue and red indicating:

Highlighted regions represent statistically significant vertices.

DISCUSSION

We have uncovered a pattern of associations of microstructural volumes across gray and white matter as well subcortical shapes with motor functioning.

In this study, we uncovered associations between cerebral microstructure and motor function beyond aggregated measures that are based on large, arbitrarily defined anatomical regions. A key strength of this study is its population-based design, which facilitates generalizability of the results to populations of similar ethnicity (primarily Caucasians). Also, we employed diffusion tensor-imaging, voxel-based morphometry and shape analysis in a large group of individuals, which allowed for parallel assessment of microstructural regions and connections across gray and white matter involved in motor functioning. Furthermore, our motor function assessment battery comprised a broad array quantitative tests of several upper and lower extremity motor functions. This study also had limitations. First, because of the cross-sectional design, we could not assess

whether microstructural deficits preceded (subtle) motor impairments. Second, the cerebellum could not be fully incorporated in the field of view of the MRI scan in all participants. Third, we only assessed motor functions under single-task conditions. Brain networks associated with motor function performance may vary under cognitive or physical interference.[23] Aside from these strengths, some methodological issues should be considered. We performed a principal component analysis with Varimax rotation on motor function parameters to ensure that motor domains were independent of each other. A potential downside to this approach is that some parameters which cluster at the population level may not be affected in the same direction in specific patient populations. In particular, we observed that higher drawing speed was correlated with worse manual precision. In patients with PD, drawing speed typically declines, while manual precision also decreases.

We observed associations between several clusters of gray matter voxels with worse performance on the Gait Pace and Gait Phases domains. The associations with Gait Phases are particularly intriguing, as this domain reflects bradykinetic gait, which is a prominent feature of PD. Gait Pace reflects gait speed and step length, which are commonly affected in PD as well as in an array of other diseases. Interestingly, the caudate nucleus, which is primarily known for its role in PD, was among the regions most distinctly associated with gait. Intriguingly, the angular gyrus, which is involved in spatial awareness, was also distinctly associated with gait. This is noteworthy because the angular gyrus is connected with the caudate nucleus,[24] and because microstructural impairment in the tract that connects these regions (inferior fronto-occipital fasciculus)[24] was also associated with gait. Furthermore, we observed distinct associations with gait of the insular cortex, which is highly interconnected with the basal ganglia and may

have an important role in sensorimotor processing (among other functions).[25] These associations remained virtually unchanged after excluding individuals with PD or parkinsonism due to other causes. Taken together, these findings suggest that α-synucleinopathy may also contribute to variability in gait among individuals free of clinical parkinsonism. Intriguingly, other regions strongly associated with Gait Phases and Gait Pace are the postcentral gyrus and the supramarginal gyrus. The postcentral gyrus harbors the primary somatosensory cortex (home to most thalamocortical projections), while the supramarginal gyrus is part of the somatosensory association cortex, which interprets tactile sensory data and is involved in perception of space and limbs location. Taken together, these associations highlight the importance of sensory feedback from somatosensory and proprioceptive circuits for the regulation of normal gait.

As for upper extremities, we observed an association of the anterior cingulate gyrus with Manual Precision. This region may have an error detection function[26] or allocate control over movement when it receives conflicting input from different regions in the brain. We also observed an association of the lateral region of the occipital lobe and lingual gyrus with Tremor, suggesting that feedback from visual processing may affect upper extremity motor function performance. Microstructural deficits in regions and connections involved in pyramidal, extrapyramidal, visual, somatosensory, and proprioceptive systems contribute to variability in motor function performance among individuals who lack a clear underlying macrostructural brain deficit.

In conclusion, microstructural deficits in regions and connections involved in pyramidal, extrapyramidal, visual, somatosensory, and proprioceptive systems contribute to variability in motor function performance among individuals free of clinical neurodegenerative disease.

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Chapter 2.3

Metabolism

2

ABSTRACT

Gait disturbance is proposed as a mechanism for higher risk of falling in kidney disease patients. We investigated the association of kidney function with gait pattern in a general population. We also investigated whether the association between impaired kidney function and falling is more pronounced in individuals with worse gait. We included 1,430 participants (mean age: 60 years) from the Rotterdam Study. Kidney function was assessed using estimated glomerular filtration rate (eGFR) and albumin-to-creatinine ratio (ACR). We assessed Global Gait, gait velocity, and seven independent gait domains: Rhythm, Phases, Variability, Pace, Tandem, Turning and Base of Support. Regression models adjusted for cardiometabolic and neurological factors were used. We evaluated whether participants with impaired kidney function and impaired gait fell more in the previous year. Study population had median [interquartile range] ACR of 3.6 [2.5-6.2] mg/g and mean±standard deviation (SD) eGFR of 87.6±15mL/min/1.73m^2. Higher ACR and lower eGFR were associated with lower Global Gait score (per doubling of ACR: -0.10, 95% confidence interval (CI): -0.14, -0.06, and per SD eGFR:-0.09, 95% CI: -0.14, -0.03) and slower gait speed (ACR: -1.44cm/s, CI: -2.12, -0.76; eGFR:-1.55cm/s, CI: -2.43, -0.67). Worse kidney function was associated with lower scores in Variability domain. The association between impaired kidney function and history of falling was present only in participants with lower gait scores (OR for ACR: 1.34 (1.09, 1.65); eGFR: 1.58 (1.07, 2.33)). In conclusion, we observed a graded association between lower kidney function and impaired gait. These data suggest that individuals with decreased kidney function, even at an early stage, need to be evaluated for gait abnormalities and might benefit from fall prevention programs.

BACKGROUND

A decline in physical functioning is a common finding in patients with chronic kidney disease (CKD).[1] Deterioration in activities of daily living begins already in early stages of CKD and progressively worsens, leading to a lower quality of life and ultimately shorter survival in these patients.[2,3] One of the key contributors in performing activities of daily living is a proper walking pattern.[4,5]

Gait is a complex process that includes various independent domains, and the underlying determinants of each domain may differ. Previous studies showed that patients with CKD more frequently experience gait abnormalities, which may lead to higher risk of falling in these patients.[6] Still, there is a scarcity of empirical data on the association between kidney function and domain-specific gait disturbances. Furthermore, it remains unclear whether the link between impaired kidney function and falling is more pronounced in individuals with worse gait.

In this population-based study, we studied the independent associations of kidney function with gait pattern and various gait domains. In addition, we investigated whether the association between kidney function and prevalence of fall is stronger in individuals with worse gait.

METHODS

Study population

The study is performed within the framework of the Rotterdam Study. The design of the Rotterdam Study has been described previously.[7] For this study, we used the third cohort of the Rotterdam Study including participants 45 years and older

living in Ommoord, a district of Rotterdam, The Netherlands. Between March 2009 and March 2011, 1,643 individuals from the third cohort were invited to undergo gait assessment. In total, gait measurements were performed in 1,509 participants (exclusions were due to physical inability, refusal, and technical problems). Given the tight link between gait and neurological disorders,[8] we further excluded 24 participants from the analyses due to history of stroke (symptomatic cerebral ischemia) (n=21), dementia (n=1), and symptoms of Parkinsonism (n=2). This resulted in 1,485 participants, of whom 1,430 had serum cystatin C data, and 1,400 had urine albumin and creatinine measurements.

Kidney function

Serum creatinine is influenced by muscle mass and muscle mass is an important component of gait. Therefore, we estimated glomerular filtration rate based on cystatin C measurement which is believed to be independent of muscle mass.[9] Serum cystatin C was measured with a particle-enhanced immunonephelometric assay. Estimated glomerular filtration rate (eGFR) was calculated for cystatin C based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[10] Participants collected the first morning urine before arriving to the research center. Urine albumin and creatinine were determined by a turbidimetric method and measured by a Hitachi MODULAR P analyzer (Roche/Hitachi Diagnostics, Mannheim, Germany). Albumin-to-creatinine ratio (mg/g) was estimated by dividing urine albumin by creatinine. Since albumin-to-creatinine ratio was not normally distributed, we used base 2 log-transformed values to obtain values per doubling of albumin-to-creatinine ratio. We added 1 to the non-transformed values to account for those who did not have albuminuria.[11]

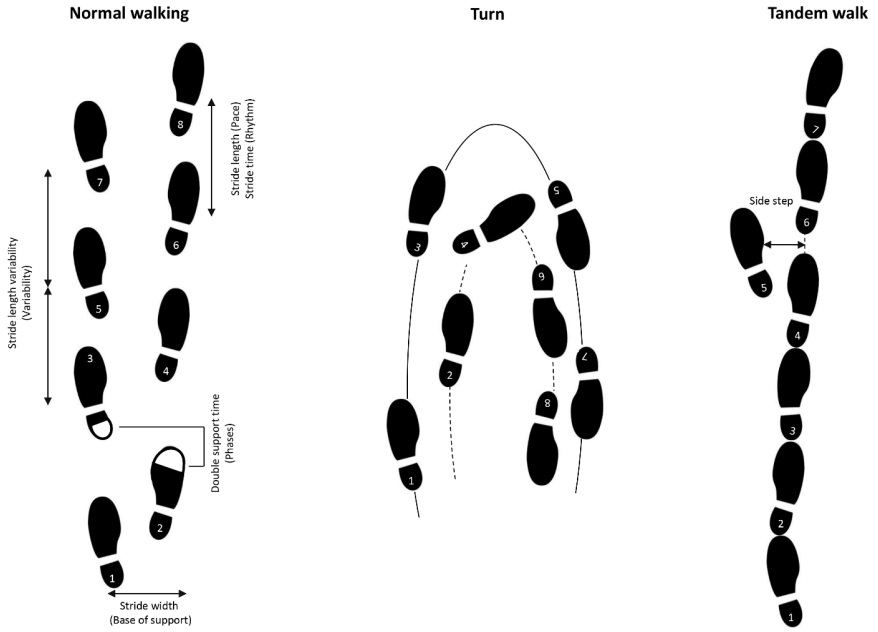
Apart from continuous measures of kidney function, we made three kidney function categories. We made the categories on the basis of two criteria: eGFR > 60 mL/min/1.73m^2 and albumin-to-creatinine ratio < 30 mg/g. First category includes participants that met both criteria. Second category included participants that met only one criterion. Participants that met none of the criteria were classified as the third category.

Gait and fall assessment

Details of the gait assessment have been published elsewhere.[5] In brief, a 5.79 m long electronic walkway with pressure sensors (GAITRite Platinum; CIR systems, Sparta, NJ: 4.88 m active area; 120 Hz sampling rate) was used to assess gait. Standardized gait protocol consists of three walking conditions: normal walk, turning and tandem walk. In 'normal walk', participants were asked to walk eight times at their usual pace across the walkway. We considered the first walk as a practice walk and did not use it for gait parameter calculations. In 'turning', participants walked across the walkway, turned halfway, and returned to their starting position. In 'tandem walk', participants were asked to walk heel-to-toe over a visible line on the walkway (*Figure 1*). Using principal components analysis, we summarized mean gait parameters of both legs into seven gait domains: 1) Rhythm, reflecting cadence and single support time (time when the body mass is carried by a single limb); 2) Variability, reflecting variability in step length and time; 3) Phases, reflecting double support time (time when both foot are in ground contact) and single support time as a percentage of the total stride time; 4) Pace, reflecting step length and velocity; 5) Tandem, reflecting errors in tandem walking; 6) Turning, reflecting the number of steps and time needed to turn; and

7) Base of Support, reflecting stride width and stride width variability **(***Figure 1* and *Online Supplementary* data*, Table S1***)**.[5] If necessary, gait domains were inverted so that lower values of the gait represent a worse gait. Subsequently, Global Gait was calculated as the z-standardized average of the seven gait domains. Since gait speed is the most commonly assessed gait parameter and has been shown to be a strong predictor of survival,[12] we additionally used gait speed from 'normal walking' (cm/s) as an additional measure of Global Gait. Fall was assessed using an interview questionnaire asking whether participants fell in the last 12 months that had serious consequences such as breaking a bone.[13]

Figure 1 | Three walking conditions and different gait domains.



Normal walk: The gait domains (in the parentheses) are defined through the highly correlating gait parameters. Turning walk: Turning time was measured as the time between the last contact of foot one to first contact of foot seven. Number of feet minus 2 was calculated as the turn step count. Tandem walk: Tandem walk was defined as the error in tandem walk; in this example the side step would be considered as the error.

Covariates

Cardiovascular risk factors: Blood pressure was measured twice on the right arm with a random zero phygmomanometer, after the participant had been seated for at least 5 min. The mean of both blood pressure values was used in the analyses. Information on antihypertensive medication use was based on home interview. Antihypertensive medications include diuretics, beta-blockers, angiotensin-converting enzyme inhibitors, and calcium channel blockers. Information related to smoking (past/current/never) was based on interviews using questionnaires. Serum total and high density lipoprotein cholesterol levels were measured using an automated enzymatic method. History of coronary heart disease was considered as a history of myocardial infarction or coronary revascularization procedures. Diabetes mellitus were ascertained using general practitioners' records (including laboratory glucose measurements), hospital discharge letters, and serum glucose measurements from the Rotterdam Study visits, which take place roughly every 4 years.

Inflammation and metabolic factors: C-reactive protein was measured in non-fasting serum samples kept frozen at 20°C by use of Rate Near Infrared Particle Immunoassay (Immage Immunochemistry System; Beckman Coulter). Hemoglobin values were measured using the COULTER AC•T diff2 hematology analyzer. Serum vitamin D concentrations measurements were performed with an electrochemiluminescence immunoassay (COBAS, Roche Diagnostics GmbH, Germany). Serum levels of calcium and phosphate were calculated using Roche/Hitachi cobas c analyser.

Locomotor factors: Instrumental activity of daily living (IADL) was assessed based on the IADL-scale by Lawton and Brody and scaled between 0 and 24.[4]

Radiographic assessment of hips and knees was performed on participants. Hip and knee osteoarthritis were scored using the Kellgren and Lawrence (K&L) grading system. Radiographic osteoarthritis was defined as a K&L-score ≥ 2.[14]

Neurological factors: Brain MRI scanning was performed on a 1.5 Tesla MRI scanner (GE Signa Excite). Scan protocol and sequence details are described extensively elsewhere.[15] In brief, an automated segmentation approach, based on the intensities of the T1-weighted, proton density-weighted and the fluid-attenuated inversion recovery scans, and a conventional k-nearest-neighbor classifier, which was extended with post-processing white matter lesion segmentation, was used to segment scans into gray matter, white matter, white matter lesion, cerebrospinal fluid and background tissue. All segmentation results were visually inspected and, if needed manually corrected. Supratentorial intracranial volume was estimated by summing gray and white matter, and cerebrospinal fluid volumes. Cortical infarcts were rated on structural sequences, and in case of involvement of cortical gray matter, they were classified as cortical infarcts. Lacunes were defined as focal hyperintensities (size ≥3 and < 15 mm) with the signal intensity of CSF on all sequences, and when located supratentorially with a hyperintense rim on fluid-attenuated inversion recovery (FLAIR) sequence. The presence of cerebral microbleeds was rated on a three-dimensional T2*-weighted gradient-recalled echo MRI scan by 1 of 5 trained research physicians, blinded to the clinical data. Apolipoprotein E (*APOE*) ε4 allele carriership was assessed on coded genomic DNA samples. We performed multiple imputation for missing data in the covariates, using a Markov Chain Monte Carlo method and used the imputed data for all the analyses.

Statistical analysis

We performed analysis of covariance where adjusted mean values of standardized scores of Global Gait were compared across different categories of kidney function based on both eGFR and albumin-to-creatinine ratio values. Mean values were adjusted for age, sex, height, and weight. We used linear regression models to investigate the associations of kidney function markers with the outcomes including Global Gait, gait speed, and different gait domains. Regression coefficients (β) and 95% CI were estimated per standard deviation (SD) increase for eGFR and per doubling of albumin-to-creatinine ratio. We adjusted all analyses for age, height, weight, and sex. In additional models we adjusted the analyses for cardiovascular risk factors (diabetes mellitus, systolic and diastolic blood pressure, smoking, and antihypertensive medication), inflammation and metabolic factors (C-reactive protein, vitamin D, calcium, phosphate, and hemoglobin levels), locomotor factors (presence of either hip or knee osteoarthritis, and IADL), and neurological factors (intracranial volume , total brain volume, microbleeds, MRI defined infarcts, white matter lesions, APOE4 carriers, and Mini-Mental State Examination (MMSE)). To improve interpretability, in an additional analysis, we log transformed Global Gait scores (a constant was added to all values to avoid missing for negative values) and reported the estimates as percentage increase in Global Gait scores. To explore whether any of the above-mentioned factors could modify the association between kidney function and Global Gait, we included an interaction term separately in each model. The interaction term was product of kidney function markers and the above-mentioned factors. Nested models were compared using F-tests. In an additional analysis, we further adjusted the associations for use of nervous system medications (N02-N07 ATC-codes). For Tandem domain, analyses were adjusted

for the step length and step count in the tandem walk. To study whether the association of kidney function measures with Global Gait and gait speed is influenced by individuals with comorbidities such as coronary heart disease, diabetes mellitus and kidney dysfunction, we repeated the analyses excluding participants with history of coronary heart disease, diabetes mellitus and eGFR<60 mL/min/1.73m^2 (n=222). To explore whether the link between kidney function and falling is through worse gait, we used logistic regression models including kidney function as an independent variable and history of falling (in the past 12 months) as an outcome in participants with high and low Global Gait scores. We divided the population to low and high Global Gait based on the median of the Global Gait scores. Effect modification was assessed by adding an interaction term in the regression model. The interaction term was the product of the kidney function measures and Global Gait scores. In the stratified analysis, we investigated the association of albumin-to-creatinine ratio and eGFR with history of falling in two groups of participants with low and high scores of Global Gait. To explore whether this association is independent of gait speed, we repeated the analyses adjusting for gait speed. All analyses were carried out using SPSS 20.0.2 for windows or R version 3.1.2.

RESULTS

Characteristics

Characteristics of the participants are shown in *Table 1* and *Online Supplementary data, Table S2*. The study population had a mean age of 59.7 ± 5 years and 57% were women. There were 45 participants with eGFR< 60 mL/min/1.73m^2 and 73 individuals with albumin-to-creatinine ratio>30 mg/g.

Table 1 (part I) | Study population characteristics.

Characteristics	n=1430
Age, years	59.7 (5.1)
Women	816 (57.1)
Cardiovascular risk factors	
Systolic blood pressure, mmHg	131.0 (18.4)
Diastolic blood pressure, mmHg	82.2 (10.7)
Antihypertensive medication	288 (20.1)
Smoking	
Current	376 (26.3)
Former	608 (42.5)
Total cholesterol, mmol/l	5.6 (1.0)
HDL cholesterol, mmol/l	1.4 (0.4)
Diabetes mellitus	121 (8.5)
Coronary heart disease	39 (2.6)
Weight, kg	80.2 (15.3)
Height, cm	170.5 (9.4)
Inflammation and metabolic factors	
Hemoglobin, mmol/l	8.8 (0.7)
C-reactive protein, mg/l	1.2 [0.6, 2.5]
Serum vitamin D, nmol/l	61.3 (27.3)
Serum calcium, mmol/l	2.5 (0.1)
Serum phosphate, mmol/l	1.1 (0.2)
Inflammation and metabolic factors	
Hemoglobin, mmol/l	8.8 (0.7)
C-reactive protein, mg/l	1.2 [0.6, 2.5]
Serum vitamin D, nmol/l	61.3 (27.3)
Serum calcium, mmol/l	2.5 (0.1)
Serum phosphate, mmol/l	1.1 (0.2)
Motor factors	
Instrumental activity of daily living, points	1.4 (2.2)
Osteoarthritis (knee/hip)	143 (10.0)

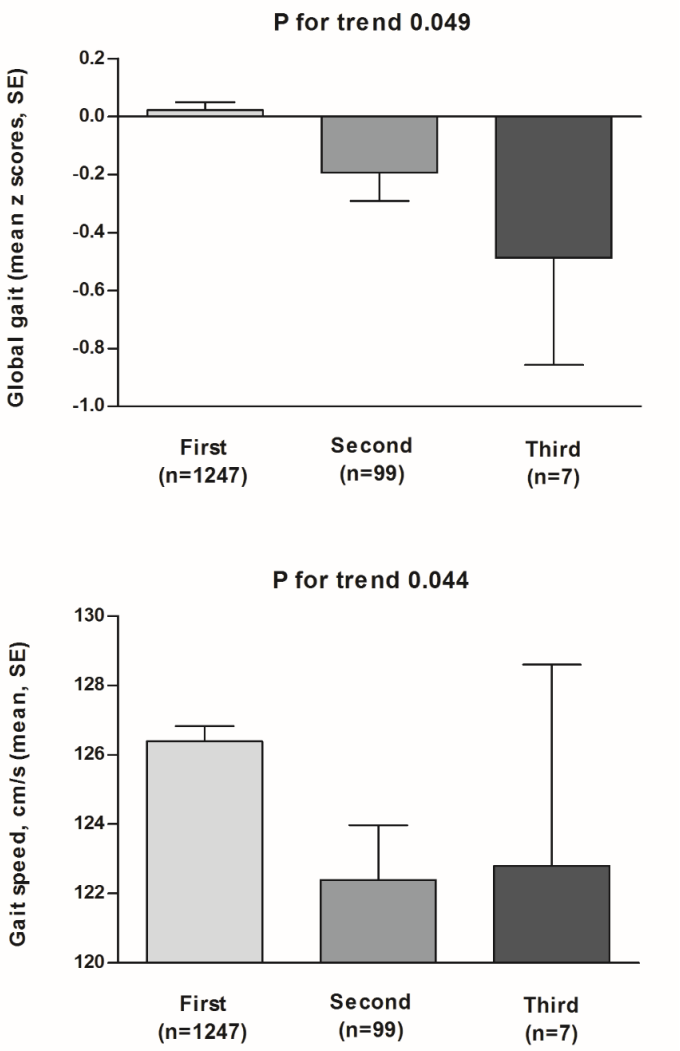
Table 1 (part II) | Study population characteristics.

Characteristics	n= 1,430
Neurological factors	
APOE4 carriers	401 (30.2)
White matter lesion volume, mL	1.9 [1.3, 3.2]
MMSE, score	29 [27, 29]
Microbleeds	149 (10.4)
Lacunar infarcts	45 (3.1)
Cortical infarcts	17 (1.2)
Intracranial volume, mL	1127.7 (121.5)
Total brain volume, mL	958.4 (102.0)
Kidney function measures	
Albumin-to-creatinine ratio, mg/g	3.6 [2.3, 6.2]
eGFR, mL/min/1.73 m^2	87.6 (14.8)

Categorical variables are presented as numbers (percentages), continuous variables as means (standard deviations) and white matter lesions and albumin-to-creatinine ratio are presented as medians (interquartile ranges).
eGFR: estimated glomerular filtration rate, MMSE: mini–mental state examination.

Figure 2 shows mean values of Global Gait and gait speed in categories of kidney function. We observed a trend (p value < 0.05) when plotting the adjusted mean values of Global Gait and gait speed across different categories of kidney function based on both albumin-to-creatinine ratio and estimated eGFR, indicating that participants with worse kidney function have worse Global Gait and slower walking speed (*Figure 2*).

Figure 2 | Adjusted mean and standard error (SE) of standardized Global Gait and gait speed values across categories of kidney function.



Mean values were adjusted for age, sex, height, and weight. First: eGFR > 60 mL/min/1.73 m2 AND albumin-to-creatinine ratio < 30 mg/g. Second: eGFR> 60 mL/min/1.73 m2 AND albumin-to-creatinine ratio > 30 mg/g OR eGFR < 60 mL/min/1.73 m2 AND albumin-to-creatinine ratio < 30 mg/g. Third: eGFR < 60 mL/min/1.73 m2 AND albumin-to-creatinine ratio > 30 mg/g.

Kidney function, Global Gait and gait speed

The association of kidney function markers with Global Gait and gait speed is presented in *Figure 3***.** In the basic model (adjusted for age, sex, height, and weight), higher albumin-to-creatinine ratio was associated with worse Global Gait (difference in standardized scores of gait per doubling of albumin-to-creatinine ratio: -0.10; 95% confidence interval (CI): -0.14, -0.06). Similarly, each SD lower eGFR was associated with 0.09 lower values of standardized z scores of Global Gait (95% CI: -0.14, -0.03). Higher albumin-to-creatinine ratio was associated with slower gait speed (-1.44 cm/s; 95% CI: -2.12, -0.76). Each SD lower eGFR was associated with slower gait speed (-1.55 cm/s; 95% CI: -2.43, -0.67). Separate adjustments for motor, metabolic, and neurological factors did not essentially change the associations. Associations attenuated after adjusting for cardiovascular risk factors. When adjusting for all factors in a single model, the effect estimates attenuated (from 2% to 1% change in Global Gait score per SD eGFR increase or doubling of albumin-to-creatinine ratio) but remained statistically significant (*Online Supplementary Table S3* and *Figure 3*). Adjusting the associations for use of central nervous system medications did not change the associations. Excluding participants with history of coronary heart disease, diabetes mellitus and eGFR<60 mL/min/1.73m^2 did not change our findings (*Online Supplementary Table S4*). No significant interaction terms were observed between kidney function markers and motor, inflammatory, metabolic, neurological and cardiovascular risk factors in relation to Global Gait or gait speed.

Kidney function and gait domains

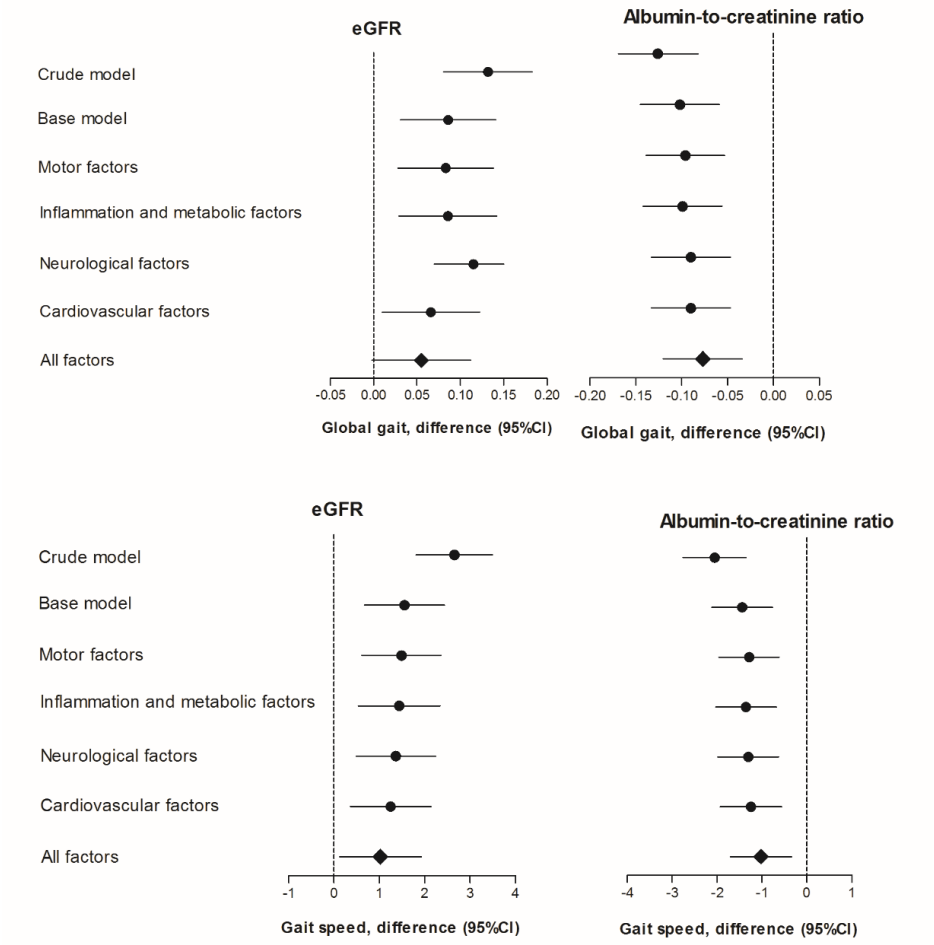
Figure 4 shows the association of kidney function markers with various gait domains. From seven gait domains, both higher albumin-to-creatinine ratio and lower eGFR were associated with lower scores of Variability (p values < 0.05) indicating that people with impaired kidney function have exaggerated gait variability. Furthermore, higher albumin-to-creatinine ratio was associated with slower scores in Pace domain and lower scores in Phase domain (all p values <0.05).

Kidney function, gait and risk of fall

In the total population, each doubling of albumin-to-creatinine ratio was associated with 1.24 higher odds of falling in the past year. Similarly, each SD (14.8 mL/min/1.73 m^2) was associated with 1.30 higher odds of falling in the past year.

When dividing the population to low and high Global Gait, we observed that in individuals with low Global Gait scores, higher albumin-to-creatinine ratio was associated with higher prevalence of falls, whereas such an association was not found in individuals with better Global Gait scores (OR: 1.34; 95% CI: 1.09, 1.65 vs OR: 1.15; 95% CI: 0.86, 1.53). This difference was statistically significant (P for interaction term = 0.035). Similarly, lower eGFR was associated with higher prevalence of falling only in participants with lower Global Gait scores (OR: 1.58; 95% CI: 1.07, 2.33 vs OR: 1.09; 95% CI: 0.74, 1.60). This difference was statistically significant (P for interaction term = 0.011) (*Table 2*).

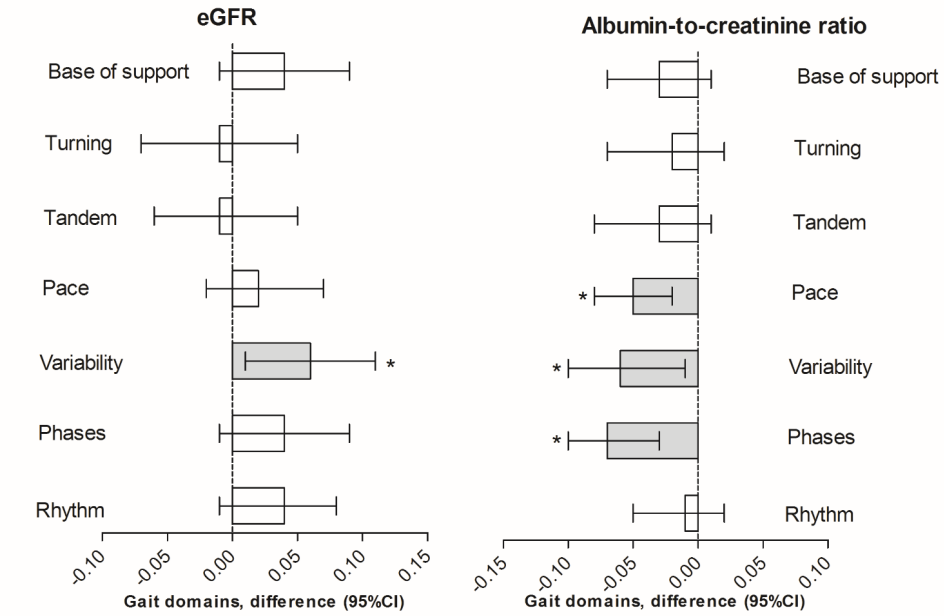
Figure 3 | Association between kidney function and Global Gait and gait speed adjusting for various factors.



Base model: Adjusted for age, sex, height, weight. Cardiovascular risk factors: Adjusted for age, sex, height, weight, diabetes mellitus, systolic and diastolic blood pressure, smoking, and antihypertensive medication. Inflammation and metabolic factors: Adjusted for age, sex, height, weight, C-reactive protein, vitamin D, calcium, phosphate, and hemoglobin. Motor factor: Adjusted for age, sex, height, weight, presence of either hip or knee osteoarthritis, and instrumental activity of daily living (IADL). Neurological factors: Adjusted for age, sex, height, weight, intracranial volume, total brain volume, microbleeds, lacunar infarct, cortical infarct, white matter lesion, APOE4 carriers, and MMSE.

Adjusting for cardiovascular risk factors, motor, metabolic, and neurological factors as well as gait speed did not change *the results (Table 2, model 2 and Online Supplementary data, Table S5). This finding suggests the association between kidney function and falling is more promin*ent in individuals with low Global Gait scores.

Figure 4 | The association of kidney function parameters with different gait domains.



*Gray bars with stars indicate statistically significant results. *Differences (regression coefficients [β]), and 95% confidence intervals are calculated using the per standard deviation increase in eGFR and subject-specific standardized z scores for various gait domains. Differences for albumin-to-creatinine ratio represent measures of gait components per doubling of the albumin-to-creatinine ratio. All analyses were adjusted for age, sex, height, weight and all the gait domains. For Tandem, analyses were adjusted additionally for step count and step size. Estimated glomerular filtration rate (eGFR), confidence interval (CI).*

Table 2 | Association of kidney function markers with prevalence of falling in categories of Global Gait.

	Odds ratio of poor kidney function for falling			
	Total population	Above-median Global Gait	Below-median Global Gait	P for interaction term
Albumin-to-creatinine ratio				
Model 1	1.24 (1.06, 1.46)	1.34 (1.09, 1.65)	1.15 (0.86, 1.53)	0.035
Model 2	1.23 (1.03, 1.48)	1.45 (1.14, 1.85)	1.08 (0.78, 1.49)	0.046
eGFR				
Model 1	1.30 (1.00, 1.69)	1.58 (1.07 2.33)	1.09 (0.74, 1.60)	0.011
Model 2	1.24 (0.93, 1.65)	1.81 (1.12, 2.93)	1.04 (0.69, 1.58)	0.016
Model 1	1.24 (1.06, 1.46)	1.34 (1.09, 1.65)	1.15 (0.86, 1.53)	0.035
Model 2	1.23 (1.03, 1.48)	1.45 (1.14, 1.85)	1.08 (0.78, 1.49)	0.046

Odds ratios are presented per each negative standard deviation of eGFR and per doubling of albumin-to-creatinine ratio, in the total population and stratified by Global Gait.

Model 1: Adjusted for age, sex, height and weight. Model 2: Additionally adjusted for diabetes mellitus, systolic and diastolic blood pressure, smoking, antihypertensive medication, C-reactive protein, vitamin D, calcium, phosphate, hemoglobin, presence of either hip or knee osteoarthritis, instrumental activity of daily living (IADL), intracranial volume, total brain volume, microbleeds, lacunar infarct, cortical infarct, white matter lesion, APOE4 carriers, and MMSE. eGFR: estimated glomerular filtration rate based on cystatin C, CI: confidence interval, OR: odds ratio.

DISCUSSION

In this population-based study, we observed that worse kidney function is independently associated with worse performance in Global Gait and slower gait speed as well as with lower scores on the Variability domain. Furthermore, in participants with lower scores of Global Gait, impaired kidney function was associated with falling.

A link between worse kidney function and decline in physical ability has been shown previously.[2,3,16,17] Investigators from The Health, Aging and Body Composition (Health ABC) Study found that individuals with eGFR less than 60 mL/min/1.73 m^2 are more likely to have slower gait speed, reduction in lower extremity performance and lower strength.[2] Consistently, in the Cardiovascular Health Study, the investigators showed that a higher level of serum creatinine is associated with lower scores in activity of daily living.[17] The Framingham Offspring Study, which included participants with mean age of 68 years, found that patients with CKD (as defined by cystatin C) experience a steeper decline in gait speed.[16] In the present study, we showed that impaired kidney function is associated with higher prevalence of falls only in participants with low scores of gait. We also found that impaired kidney function was most prominently related to worse Variability, which previously have been shown to be specifically related to higher risk of falls.[18] Given the high occurrence of falls in patients with kidney disease,[6] gait abnormalities could be considered as potential predisposing factors for higher risk of falling in these patients. Some gait domains, such as Variability, are difficult to assess visually, hence a thorough computerized gait assessment is potentially a useful tool to identify patients most in need of fall prevention programs.

We considered several underlying factors which may explain the association between kidney function and gait. In particular, impairment in kidney function is known to be related to osteoarthritis,[19] inflammation and metabolic factors,[20,21] patients with kidney disease are at increased risk of cerebrovascular and neurodegenerative disorders[11] which are tightly linked to gait,[8] and cardiovascular risk factors such as smoking, high blood pressure, and diabetes are related to both impairment in kidney function and gait deterioration. Adjusting for these factors separately and including them in a model as an interacting factor did not fully explain the associations between kidney function and gait.[4,22] From all the factors, adjusting for cardiovascular factors attenuated the association between kidney function and gait the most, suggesting that the relation could be partly explained by cardiovascular risk factors. Another explanation for the association between impaired kidney function and gait could be the direct impact of kidney function on gait. Impairments in kidney function, even in subclinical stages, is associated with the accumulation of neurotoxins that have been shown to cause axonal loss with secondary or predominant demyelination, β-2-microglobulin deposition in joints and connective tissue, and increased levels of inflammatory cytokines which can all result in muscle mass reduction, muscle strength deterioration, and loss of balance.[23,24]

Strengths of our study include the relatively large sample size and availability of extensive information on various kidney function parameters and different neurological and cardio-metabolic risk factors which enabled us to control for several potential factors that can influence the association between kidney function and gait. Several limitations of our study also merit attention. First, gait was assessed in a research setting and it is possible that people with severe gait problems are under-represented in a population-based study. Nonetheless, our

findings indicate that lower kidney function is associated with more subtle gait abnormalities before full-blown gait abnormalities become evident. Second, given the cross-sectional design of this study, we cannot definitively establish the temporal relationship of the association between kidney function, gait and falling. As a consequence, it is possible that in some individuals, falls resulted in gait problems, which in turn led to a decline in overall health and subsequently in a diminishing kidney function. Still, we consider it unlikely that such a cascade would have driven our main observations. Third, cystatin C levels and spot albuminuria may vary within individuals over time and therefore a single measurement of these markers may be an imperfect metric of kidney function.

In conclusion, we show that worse kidney function is associated with slower gait speed and a worse gait pattern. Low eGFR and high albumin-to-creatinine ratio were each associated with lower scores on the Variability domain. Our findings suggest that kidney function, even in subclinical stages, is associated with impairments in gait, which in turn may increase the risk of falling and potentially lead to disability and a reduced quality of life.

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Chapter 3

Motor functioning: association with neurodegenerative diseases

The intellect is at first unaffected, but gets weakened at last.

Trousseau, A.
Tremblement senile et paralysis agitans. Clinique médicale de l'Hôtel-Dieu de Paris. 1865.
[In English: Senile trembling and paralysis agitans. Lectures in Clinical Medicine Delivered at the Hotel Dieu, Paris. 1868, London: New Sydenham Society.]

Chapter 3.1

Lifetime risk

ABSTRACT

There is a scarcity of robust data on the burden of common neurological disease at the population level. We aimed to quantify the lifetime risk of common neurological disease in older adults, including their co-occurrence and preventive potential. Within the population-based Rotterdam Study, we studied lifetime risk of dementia, stroke, and parkinsonism between 1990 and 2016. Among 12,102 individuals (57.7% women) aged ≥45 years free from these diseases at baseline, we studied co-occurrence, and quantified the combined and disease-specific remaining lifetime risk of these diseases at various ages for men and women separately. We also projected effects on lifetime risk of hypothetical preventive strategies that delay disease onset by one, two or three years. During follow-up (156088 person-years), 1,489 individuals were diagnosed with dementia, 1,285 with stroke, and 263 with parkinsonism. Of these individuals, 438 (14.6%) were diagnosed with multiple diseases. Women were almost twice as likely as men to be diagnosed with both stroke and dementia during their lifetime. The lifetime risk for any of these disease at age 45 was 48.2% (95% CI 47.1%;51.5%) in women, and 36.2% (35.1%;39.3%) in men. This difference was driven by a higher risk of dementia as the first manifesting disease in women than in men (25.9% versus 13.7%; $p<0.001$), while this was similar for stroke (19.0% versus 18.9% in men) and parkinsonism (3.3% versus 3.6% in men). Preventive strategies that delay disease onset with one to three years could theoretically reduce lifetime risk for developing any of these diseases by 20% to 50%. We conclude that one in two women and one in three men will develop dementia, stroke, or parkinsonism during their life. These findings strengthen the call for prioritising the focus on preventive interventions at population level which could substantially reduce the burden of common neurological diseases in the ageing population.

BACKGROUND

Dementia, stroke, and parkinsonism are among the leading causes of mortality and disability in older individuals, and pose a huge burden on patients and their caregivers.[1] These common neurological diseases share many risk factors and subsequently tend to show substantial co-occurrence, with stroke and parkinsonism patients at increased risk of dementia, and patients with dementia at increased risk of stroke.[2,3] Recent estimates indicate that the global costs-of-illness for these diseases sum up to more than 2% of the annual world gross domestic product.[4-6] This socio-economic burden is expected to grow steeply with the ageing of populations and continuing increases in life-expectancy worldwide.[1] As a result, this has led to widespread calls for prioritising these diseases on the global health agenda.[7-9] Yet, these common neurological diseases remain understudied in terms of prevention at the population level,[10,11] and underfunded compared to other common diseases such as cancer and heart disease, which likely reflects skewed societal perceptions of lifetime risk.[12,13] While informative risk estimates exist to characterize the burden of cancer and heart disease in the population, these numbers are lacking for these common neurological diseases. Such numbers are particularly suited to use in health campaigns to raise public awareness, with the lifetime risk of one in eight for breast cancer and the risk of one in four to die from heart disease as two key examples.[14,15]

Prior studies estimating the burden of these neurological diseases, relied on prevalence or incidence rates, without appropriately accounting for their potential co-occurrence and competing non-neurological mortality.[1,4-6] To prevent potential overestimation, lifetime risk estimates can be used to reliably estimate this burden while taking into account the substantial co-occurrence of these

diseases at old age,[16] a high competing risk of mortality, as well as potential differences in life-expectancy between men and women. These lifetime risk estimates are needed as these may benefit the awareness of the burden in the population, and subsequently can be used to model what impact preventive interventions could have on this disease burden.[8,9,17-19]

In this study, we used long-term follow-up data from the population-based Rotterdam Study to study occurrence and co-occurrence of dementia, stroke, and parkinsonism in middle-aged and elderly men and women. We additionally calculated the corresponding combined, and disease-specific lifetime risk of these diseases within a competing risk framework, and studied their preventive potential.

METHODS

Study design, setting, and population

This study was performed within the Rotterdam Study, a prospective population-based cohort designed to study the occurrence and determinants of age-related diseases in the general population. Details regarding the objectives and design have been reported previously.[16] Briefly, in 1990 all inhabitants aged 55 and older from a well-defined suburb in the city of Rotterdam, the Netherlands. This initial cohort comprised 7,983 individuals. In 2000, 3,011 individuals who had become 55 years of age or moved into the study district since the start of the study if aged 55 years and older, were added to the cohort. In 2006, a further extension of the cohort was initiated in which 3,932 individuals were included, aged 45 years

and older. In total, the Rotterdam Study comprises 14,926 individuals aged 45 years or over. The overall response rate for all three recruitment waves was 72%.

Because the aim was to determine the risk of developing disease, we excluded individuals who already had a history of these diseases at baseline (N=1,019: dementia [N=420], stroke [N=378], parkinsonism [N=106], or with a history of a combination of these diseases [N=115]). We additionally excluded individuals who were insufficiently screened at baseline for at least one of these diseases (N=1,780). We further excluded individuals who did not provide informed consent to access medical records or hospital discharge letters (N=25), leaving 12102 individuals available for analyses.

Ascertainment methods of dementia, stroke, and parkinsonism

Baseline and follow-up ascertainment methods for dementia, stroke and parkinsonism have previously been described in detail and are summarized in *Online Supplementary Appendix A*. At baseline, disease ascertainment comprised extensive structured interviews, examinations, and information from medical records, hospital discharge letters, and pharmacy data to ensure that participants were free of any of these diseases. During follow-up, we screened for these diseases during repeated examinations and interviews every four years. We additionally ensured continuous monitoring for disease through computerized linkage of medical records from general practitioners and the regional institute for outpatient mental healthcare with the study database. In the Dutch healthcare system, the entire population is entitled to primary care that is covered by their obligatory health insurance. The general practitioner functions as a gatekeeper for referral to secondary and tertiary care providers, who report back to the referring

general practitioner about test results and clinical diagnoses. With this linkage, the entire cohort is thus continuously monitored for detection of new cases or possible clinical signs of these diseases between center visits.

Of all individuals who were screened and suspected of having any of these diseases, case reports were drawn up covering all potentially relevant information to establish the presence of disease. These case reports were evaluated by a consensus panel led by a consultant neurologist to adjudicate the final diagnosis in accordance with standardized diagnostic criteria which were held constant over the entire follow-up time (DSM-III-R for all-cause dementia and NINCDS–ADRDA for Alzheimer's Disease), stroke was defined according to WHO criteria, and parkinsonism including Parkinson's Disease (PD) were defined according to strict study criteria (additional information in *Online Supplementary Appendix A* and **Chapter 4.1**). In addition, available clinical neuroimaging data were used if required to determine the subtype of dementia. The subtype of stroke was classified as unspecified if no imaging was available. Study follow-up ended at incident outcome diagnosis, death, or 1 January 2016, whichever came first. Follow-up was virtually complete (96.2% of potential person-years).

Ascertainment methods of study population characteristics

Standardized assessment of anthropometrics, risk factors, and use of medication at baseline through in-person examinations at the research center, home interviews, and laboratory assessments is described in *Online Supplementary Appendix B*.

Statistical analysis

Preclusion of disease-specific outcomes of interest by death or precluding events is referred to as competing risks.[20] Due to these competing events, absolute risks are overestimated in standard Kaplan-Meier analyses. Since women on average live longer than men, this overestimation will be differential. To overcome these issues, we analysed the data taking into account the occurrence of competing events to compute remaining lifetime risks in left truncated data with age as time scale, while stratifying by sex in all analyses. Lifetime risk estimates reflect the competing risk-adjusted cumulative incidences from that particular age to the age of last observation. In this study, the maximum age was 106 years for men and 107 years for women.

First, we studied occurrence and the co-occurrence of these diseases during follow-up. We quantified the number of events for each disease separately, and visualized their co-occurrence among individuals using Venn diagrams.

Second, we calculated the combined and disease-specific lifetime risk. For these analyses, follow-up started at study entry (with the age of 45 years as minimum) and ended at the first date of diagnosis of any of the three diseases. This meant that we considered only the event of the three potential outcomes, whichever occurred first. For instance, individuals who first experienced a stroke during follow-up were no longer at risk of dementia or parkinsonism. Within this framework, we assessed the combined cumulative incidence, or risk, of these diseases from the age of 45 to the age of last observation. This equals the combined remaining lifetime risk of developing any of these diseases at the age of 45. We subsequently repeated this analysis for each disease separately, resulting in disease-specific lifetime risks of first manifestation.

Third, we repeated these analyses by changing the age of entry (from 45 to 55, 65, 75, and 85 years and older), to study whether the disease-specific lifetime risks of first manifestation differed across various ages.

Fourth, we studied disease-specific lifetime risk by considering only the disease of interest as outcome, such that individuals remained at risk of these three diseases irrespective of the occurrence of a first event. This meant that individuals with a stroke or parkinsonism during follow-up in the dementia analysis, remained at risk of dementia.

Fifth, we repeated these analyses considering only the most common subtypes for each disease separately (including Alzheimer's Disease and vascular dementia; and ischemic, haemorrhagic, and unspecified stroke; and PD).

Finally, we modelled the effects of a delay in disease onset on the combined lifetime risk of any disease and for each disease separately at index ages 45, 55, 65, 75, and 85 by postponing the date of diagnosis of all three diseases with 1, 2, and 3 years. In these analyses, we assumed a constant life-expectancy. We used nominal significance levels to compare age and sex differences ($p<0.05$). Data were handled and analysed with SPSS Statistics version 24.0.0.1 (IBM Corp., Armonk, NY) and R, CRAN version 3.4.3 (rms, etm, and cmprsk packages).

RESULTS

Population characteristics are presented in *Table 1*. Median age at baseline was 62.2 years (range 45-107 years), and women represented 57.7% of the population.

Table 1 | Study population characteristics.

	All individuals (N=12102)	Men (N=5120)	Women (N=6982)
Age in years, mean (SD)	64.4 (9.4)	63.8 (8.8)	64.9 (9.8)
Educational level, n (%)			
Primary	1943 (16.1%)	577 (11.3%)	1366 (19.6%)
Lower	4820 (39.8%)	1494 (29.2%)	3326 (47.6%)
Further	3306 (27.3%)	1844 (36.0%)	1462 (20.9%)
Higher	1848 (15.3%)	1144 (22.3%)	704 (10.1%)
Smoking, n (%)			
Never	3894 (32.2%)	723 (14.1%)	3171 (45.4%)
Past	5256 (43.4%)	2898 (56.6%)	2358 (33.8%)
Current	2871 (23.7%)	1476 (28.8%)	1395 (20.0%)
Systolic blood pressure in mmHg, mean (SD)	138 (22)	139 (21)	137 (22)
Diastolic blood pressure in mmHg, mean (SD)	77 (12)	78 (12)	76 (12)
Hypertension, n (%)	6481 (53.6)	2744 (53.6)	3737 (53.5)
Depression, n (%)	801 (6.6%)	195 (3.8%)	606 (8.7%)
Atrial fibrillation, n (%)	599 (4.9%)	309 (6.0%)	290 (4.2%)
Type 2 Diabetes, n (%)	1124 (9.3%)	574 (11.2%)	550 (7.9%)
Total cholesterol, mmol/L	6.18 (1.24)	5.91 (1.18)	6.39 (1.24)
High-density lipoprotein cholesterol, mmol/L	1.38 (0.39)	1.22 (0.33)	1.49 (0.40)
Hypercholesterolemia, n (%)	5489 (45.4)	1842 (36.0)	3647 (52.2)
APOE genotype, n (%)			
ε2/ε2 or ε2/ε3	1510 (12.5%)	605 (11.8%)	905 (13.0%)
ε3/ε3	6675 (55.2%)	2874 (56.1%)	3801 (54.4%)
ε2/ε4, ε3/ε4, or ε4/ε4	3239 (26.8%)	1400 (27.3%)	1839 (26.4%)

SD: standard deviation, APOE Apolipoprotein E.

During 156,088 person-years of follow-up, 3,037 events occurred: 1,489 individuals were diagnosed with dementia (79.7% Alzheimer's Disease), 1,285 with stroke (64.7% ischemic, 9.8% haemorrhagic stroke, and 25.4% unspecified stroke) and 263 with parkinsonism (50.6% PD). A complete overview of the occurrence of all subtypes of disease is presented in *Table 1* in Appendix C of the *Online*

Supplementary material. In total, 5,291 individuals died during follow-up, of whom 3,260 died free of these diseases. Individuals who were diagnosed with one of these diseases during follow-up, particularly tended to have a higher prevalence of hypertension (61.5% versus 51.4%) atrial fibrillation (7.7% versus 4.2%), hypercholesterolemia (56.0% versus 42.5%), and more often had type 2 diabetes (11.2% versus 8.8%) at baseline, compared to others (*Online Supplementary Table 2, Appendix C*).

Disease co-occurrence

Among those who developed one of these three neurological diseases, most individuals were diagnosed with only one of these diseases (*Figure 1*). Yet, there is a substantial risk at the age of 45 to face multiple diseases during the remaining lifespan. Among those diagnosed with one of these neurological diseases, 438 (14.6%) individuals were diagnosed with more than one disease, with women more likely to be diagnosed with more than one of these diseases during lifetime compared to men (*Figure 1*: 4.0% compared to 3.1% respectively, $p<0.001$). This difference was predominantly driven by a greater probability of overlap between dementia and stroke (occurring in 2.9% of women; 1.9% of men, $p<0.001$).

Lifetime risk of any disease

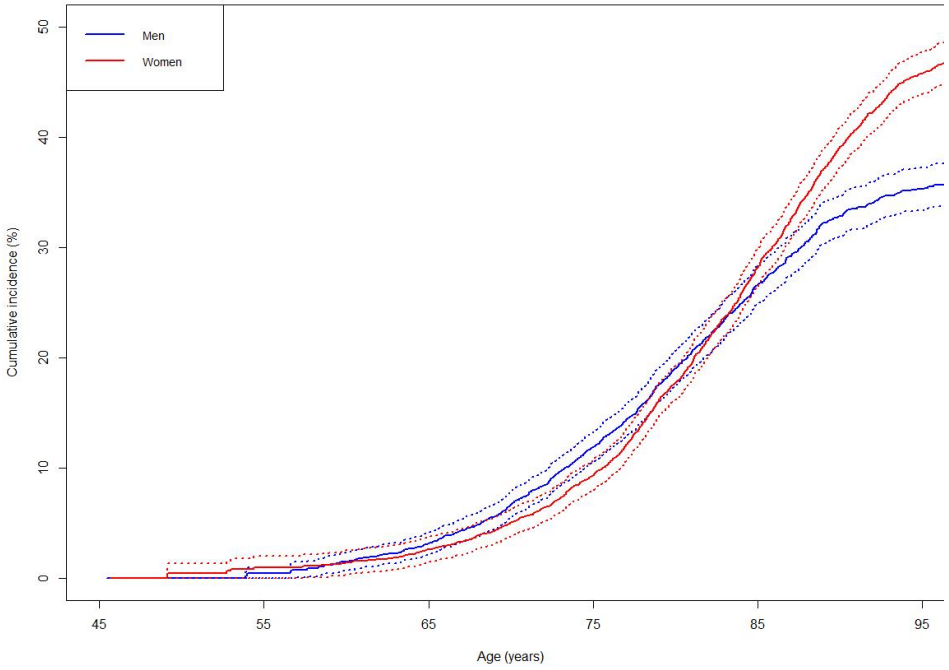
Among all individuals free of dementia, stroke and parkinsonism at baseline (N=12102), a total of 2,571 individuals were diagnosed with one of these three diseases as first manifestation: 1,245 were diagnosed with dementia, 1,118 individuals with stroke, and 208 with parkinsonism.

In *Figure 2*, the combined cumulative risk of developing any of these diseases from the age of 45 until various ages is presented for women and men separately. This risk increased steeply with age, ranging from 2.6% for women and 3.2% for men aged 45 years until age 65, to up to 45.8% and 35.3% until the age 95, respectively. The overall remaining lifetime risk of developing any of these diseases for a 45-year old woman was 48.2% (95% confidence interval (CI) 47.1% to 51.5%), while for a 45-year old man this risk was 36.3% (35.1% to 39.3%, P for sex difference<0.001).

Figure 1 | Disease co-occurrence in men and women.

Venn diagrams showing patterns of disease co-occurrence in men and women quantified in the number of events during follow-up. 4% of women and 3% of men were diagnosed with more than one of these diseases

Figure 2 | Risk of common neurological diseases.



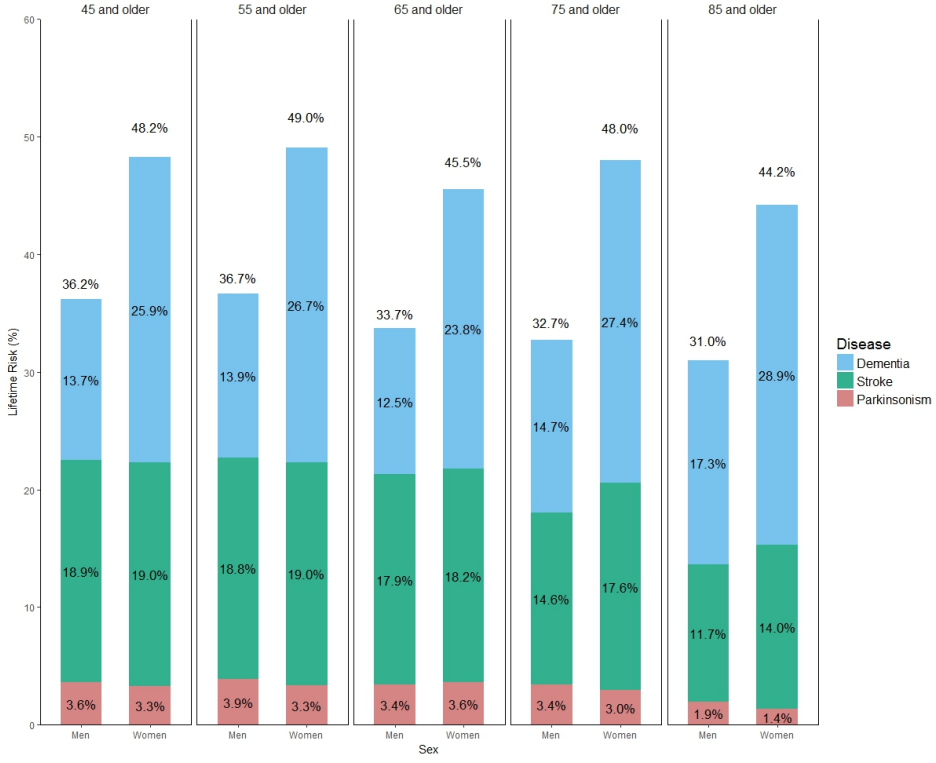
In this analysis, follow-up ended at time of first occurrence of dementia, stroke, or parkinsonism. For instance, for individuals who first suffered a stroke and subsequently developed dementia, only the stroke event is considered here.

Lifetime risk of any disease across age

Overall lifetime risk of these common neurologic diseases was stable for both men and women between ages 45 and 85 (*Figure 3*). At age 45, first manifestation of stroke posed the highest lifetime risk for men (18.9%). Dementia posed the largest risk for women (25.9%), which was significantly higher compared to that for men (13.7%; P for sex difference<0.001). This sex difference remained largely stable across all index ages (p<0.001 for all index ages). *Figure 3* also shows that with advancing age, the relative contribution of dementia to the remaining lifetime risk of any disease increased in both men and women, representing

66.6% of all first diagnoses in elderly women (i.e. >85 years) and 55.6% in elderly men. For stroke, men and women had similar lifetime risk at the age of 45 (18.9% in men compared to 19.0% in women, p=0.46). However, men were at substantially higher risk of developing stroke at younger ages, such that they have a 8.4% risk of developing stroke before the age of 75 years compared to 5.8% for women (p=0.005). Lifetime risk of parkinsonism peaked earlier compared to dementia and stroke, was low after 85 years, and not significantly different between men and women at any age (P>0.16).

Figure 3 | Remaining lifetime risk of first manifestation of common neurological diseases at different ages, stratified by sex.



Lifetime risk of each disease separately

When individuals remained at risk of all diseases irrespective of the occurrence of precluding events, women aged 45 years had a significantly higher lifetime risk of developing dementia and stroke than men (31.4% compared to 18.6% in men; p<0.001, and 21.6% compared to 19.3% in men; p=0.03, respectively), whereas lifetime risk of parkinsonism (4.3% in women and 4.9% in men, p=0.28) was not significantly different (*Figure 4*).

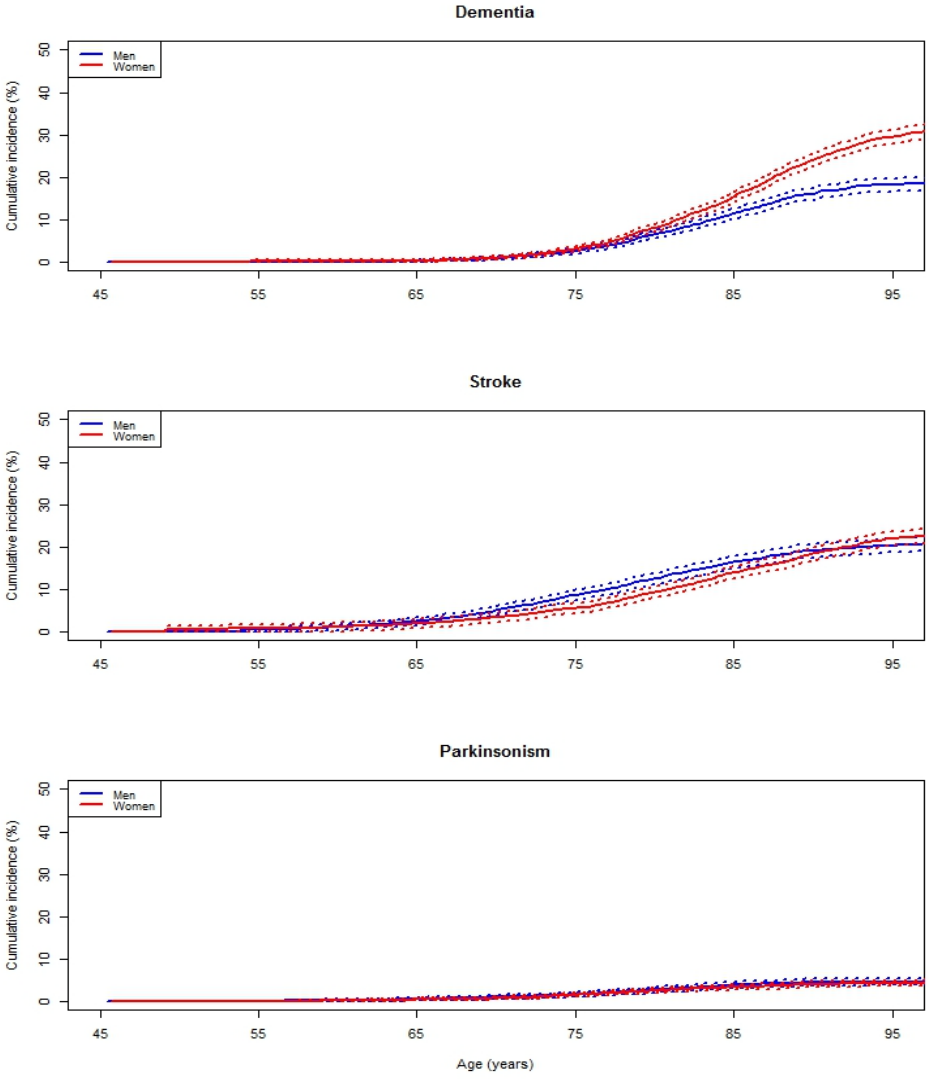
We observed similar patterns in sex-specific occurrence for Alzheimer's Disease and vascular dementia, and ischemic, haemorrhagic, and unspecified stroke, and PD (*Appendix C*, *Online Supplementary Figure 1*, and *Table 3*).

Projecting a delay in disease onset and occurrence

When projecting a delay in disease onset of one, two or three years for all three diseases, the remaining lifetime risk of these common neurological diseases could be reduced by 20% in individuals aged 45 years and older, and by more than 50% in the oldest of old (*Figure 5*). Even a delay in onset for a few years of only one disease, could already result in substantial reductions for the combined lifetime risk of developing any of these diseases. For instance, delaying dementia onset with three years, has the potential to reduce lifetime risk of any disease by 15% for men and women aged 45, up to 30% for those aged 85 years and older. For a 85-year old woman, this lowers the risk of developing dementia during her remaining lifetime from 30.4% to 21.3%.

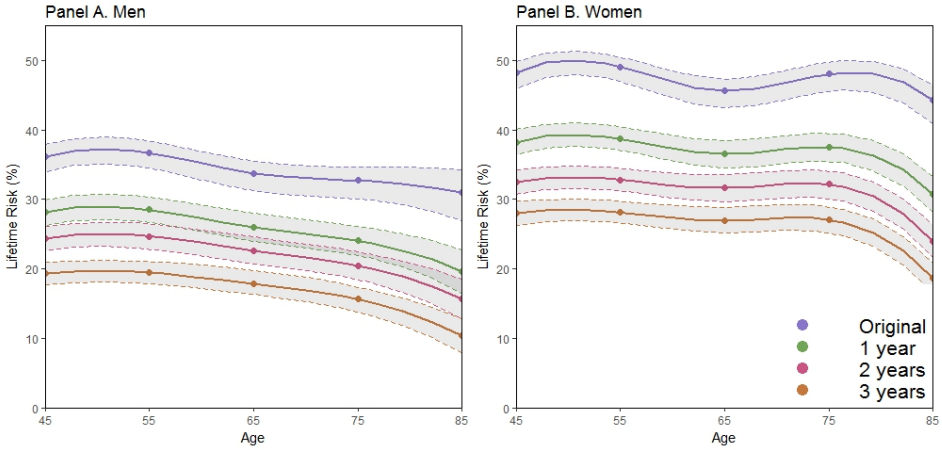
Figure 4 | Risk of dementia, stroke, and parkinsonism for 45-years-old men and women.

z



In these analyses, individuals remained at risk of each disease irrespective of the occurrence of another disease. For instance, individuals with a stroke or

Figure 5 | Modelling the effect of a 1, 2, and 3-year delayed onset of disease.



Modelling the effect of a 1, 2, and 3-year delayed onset of disease on the combined remaining lifetime risk of developing dementia, stroke or parkinsonism in men (Panel A) and women (Panel B) at ages 45, 55, 65, 75, and 85 and older. In these analyses, date of diagnosis of all three diseases was delayed with 1, 2, or 3 years while assuming a constant life-expectancy. Areas in shaded gray represent 95% confidence intervals.

DISCUSSION

In this population-based study with long-term follow-up, we found that one in two women were diagnosed with dementia, stroke, or parkinsonism during their lifetime, whereas for men this risk approximated one in three. Moreover, the risk of combined disease was higher in women than in men, with women almost twice as likely to be diagnosed with both stroke and dementia during their lifetime. These findings illustrate that lifetime risks of these diseases are very high. We further show that preventive strategies that delay disease onset of all three diseases by one to three years have the potential to reduce these risks by 20% to 50%. These findings strengthen the call for focus on prevention to reduce the

current and projected burden of common neurological diseases in the ageing population.

Methodological considerations

Several methodological considerations should be taken into account when interpreting these lifetime risks. First, this population-based study predominantly included individuals of European ancestry (97%), with a relatively long life-expectancy.[21] Generalising these lifetime risk estimates to other ethnicities or to populations with different life-expectancies should therefore be done with caution. Second, although the overall response rate in the Rotterdam Study was high (72%), non-responders and individuals with insufficient screening at study entry may have had higher than average risk factor burden and associated risk of these diseases, which may have led to some under- or overestimation of lifetime risks.[22] The direction of this effect is balancing on the impact of such risk factor burden on disease risk on the one hand, and the impact on the underlying life-expectancy of these individuals on the other hand. Third, we acknowledge that the parkinsonism syndrome has a broad spectrum of underlying causes, ranging from PD (representing 50.6% of all parkinsonism cases in this study) to reversible causes such as drug induced parkinsonism (representing only 9.7%). Fourth, we were unable to take into account severity of clinical disease, and in particular for interpretation of stroke estimates, it should be noted that about half of ischemic strokes at the population level classify as minor according to the NIH Stroke Scale (NIHSS) and may have a limited impact on daily life.[23] Strengths of this study include simultaneous assessments of these diseases using virtually complete long-term follow-up data in a large, unselected general population.

Previous studies

So far, the burden of these diseases in terms of lifetime risk were largely quantified separately,[24-29] or were determined based on prevalence or incidence rates, while not appropriately accounting for their potential co-occurrence and competing non-neurological mortality.[1] This pattern of co-occurrence and competing mortality hampers reliable calculation of the risk of developing any of these diseases when applying a lifetime perspective, such that the occurrence of one disease (e.g., stroke) precludes consideration of any subsequent event (e.g., post-stroke dementia). Similarly, several risk factors (e.g., hypertension) not only increase the susceptibility for these common neurological diseases, but are also associated with an increased risk of dying from other causes (e.g., heart disease).

Previously, the Framingham Heart Study reported lifetime risks of both dementia (1 in 5 women, 1 in 10 men),[26,29] and stroke (1 in 5 women, 1 in 6 men),[24] as well as a combined estimate of those two assessed in a single study (1 in 3).[25] Compared to findings from that study, we observed higher a lifetime risk of dementia (1 in 3 women, 1 in 5 men). This discrepancy may be due to the fact that individuals in the Rotterdam Study, have a longer life-expectancy (the Netherlands, women 83.5 and men 81.7 years) compared to individuals in the Framingham Heart Study (US, women 81.2 and men 76.4 years). Apart from a longer life-expectancy in general, these findings may be explained by smaller differences in life-expectancy between men and women in the Netherlands (1.8 years), compared to the US (4.8 years). With longer life-expectancy, individuals in this study simply had more time to develop these diseases in a timeframe with high age-specific incidence rates.[21] Additionally, women in this study were substantially lower educated compared to men, which may have led to a lower dementia resilience in women. In line with estimates from the Framingham Heart

Study, we found a slightly higher lifetime risk of stroke for women compared to men. Although men have in general a greater propensity of developing a stroke before the age 90, stroke rates for women are higher at the extremes of the age distribution with an increased risk of developing ischemic stroke for those aged <65 years old and a higher risk of unspecified strokes for women among the oldest-old. Although men in this study had more adverse levels of stroke risk factors at baseline, recent evidence shows that stroke rates among women are catching up with those from men due to recent increases in the prevalence of several stroke risk factors, particularly among young women, including hypertension, smoking, and drug abuse.[30]

For parkinsonism and PD, we found similar lifetime risks for men and women, corroborating evidence from a previous study.[27] By contrast, we did find lower risks of PD for men (2.9%), compared to the lifetime risk of 6.7% reported in another study solely conducted in male physicians.[28] In part, this may reflect high diagnostic awareness among physicians, who may recognize symptoms early on. However, given the elaborate, multimodal detection methods used in the Rotterdam Study, we find it unlikely that there was underdiagnosis on a large scale in our study. Alternatively, the discrepancy may be due to differences in baseline characteristics, such as a lower history of ever-smoking (50% compared to 88% in this study), a factor that is inversely associated with the risk of PD.[31]

Implications and future directions

Lifetime risk estimates can be used to effectively inform policy makers and communicate risks to the general population, given their easier interpretation compared with measures such as incidence rates, prevalence, or relative risk.[32,33]

These approaches to raise awareness and inform the public through lifetime risk estimates have been successfully implemented for other diseases, such as breast cancer or heart disease.[14,15] Nowadays, preventive measures for primary prevention of cardiovascular disease are tailored to individual lifetime risk estimates.[34] This could inform future prevention programs for common neurological diseases. To expand on our study, lifetime risk estimates for these diseases across different ethnicities are warranted. Additionally, it would be of particular interest for future studies to further study the effects of (epi) genetic or lifestyle factors that could impact the lifetime risk of these diseases. For this public policy planning perspective, future studies with an even broader age span could expand on the current study by also targeting other common neurological diseases such as multiple sclerosis or polyneuropathy.

There are currently no disease modifying drugs available for dementia and most causes of parkinsonism, and prevention of stroke is hampered by suboptimal adherence to effective preventive strategies or unmet guideline thresholds.[35] Yet, a delay in onset of these common neurological diseases by merely a few years could reduce the population burden of these diseases substantially. In fact, preventive interventions may also contribute to a drop in competing mortality by affecting lifetime risk of diseases with similar risk factors. For instance, preventive interventions resulting in a delay in onset of strokes are likely also able to delay morbidity and mortality due to coronary or peripheral artery disease. Our projections of the preventive potential, which assumed constant life-expectancy, may therefore be overestimating the compression of morbidity, and must therefore be interpreted as the upper limit of this preventive potential. In particular with diseases that are most common in the elderly, increased life expectancy due to for example risk factor control may counterbalance lowering in

incidence rates.[36] Nevertheless, they illustrate that risks could drop strikingly with relatively minor delays in the occurrence and onset of disease, underlining the importance of preventive strategies as the way forward to combat these diseases on a global scale.[5,37-41] Recent observations on declining dementia incidence trends from several, large population-based studies in high-income countries may in fact reflect the (initial) signs of these preventive strategies through better vascular risk factor management, improved educational attainment, or other public health developments that improved the resilience for dementia.[42-44] These results also have implications for the type of prevention strategies to develop, including population-wide interventions targeted at risk factors with considerable sex-specific differences in attributable risk, such as loneliness and depression in women or diet in men.[19,38]

Conclusions

One in two women develops dementia, stroke, or parkinsonism in their lifetime, whereas this risk approximates one in three for men. Women are almost twice as likely as men to be diagnosed with both stroke and dementia during their lifetime. Risks are theoretically highly amendable by preventive interventions at the population level. These findings strengthen the call for focus on preventive interventions to reduce the burden of common neurological disease in the ageing population.

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Chapter 3.2

Manual dexterity

ABSTRACT

Early identification of individuals at high risk of developing neurodegenerative diseases is essential for timely preventive intervention. However, simple methods that can be used for risk assessment in general practice are lacking. Within the population-based Rotterdam Study, we used the Purdue Pegboard Test (PPT) to assess manual dexterity in 4,856 individuals (median age 70 years, 58% women) free of parkinsonism and dementia between 2000 and 2004. We followed these individuals until 1 January 2012 for the onset of neurodegenerative diseases (defined as first diagnosis of parkinsonism or dementia). We determined the association of PPT scores with incident neurodegenerative disease, adjusting for age, sex, study cohort, level of education, smoking, preferred hand, parental history, memory complaints and Mini-Mental State Examination. Furthermore, we determined the incremental predictive value of PPT, expressed as change in risk classification and discrimination. During follow-up (median 9.2 years), 277 participants were diagnosed with a neurodegenerative disease (227 with dementia, 50 with parkinsonism). Lower PPT scores were associated with higher risk of incident neurodegenerative diseases (hazard ratio HR=1.28, 95% confidence interval [1.18; 1.41]) and improved discrimination of incident neurodegenerative diseases. We also observed significant associations of PPT scores separately with incident dementia (HR=1.25 [1.14; 1.39]) and incident parkinsonism (HR=1.41 [1.19; 1.67]). We conclude that a rapid, non-laboratory test of manual dexterity may help to identify individuals at high risk for neurodegenerative diseases. This highlights the importance of motor function in the prediagnostic phase of both dementia and parkinsonism, and may aid in selecting individuals for refined screening and neuroprotective trials.

BACKGROUND

Neurodegenerative diseases such as Alzheimer's Disease (AD) and Parkinson's Disease (PD) constitute a major social and economic burden on the population. Due to a rapidly ageing population, this is projected to quadruple during the coming decades,[1,2] unless preventive or curative treatments can be established. However, development of disease-modifying therapies is hampered by the advanced pathological disease stage at which patients with a neurodegenerative disease receive a clinical diagnosis.[3] Therefore, measures that allow early diagnosis or risk stratification early in the disease process are urgently needed.

Development of simple, non-laboratory algorithms to identify community-dwelling individuals who are at high risk of neurodegenerative diseases would enable referral of these individuals to a neurologic or geriatric clinic for further clinical work-up for AD, PD and other neurodegenerative diseases. Subsequently, individuals at highest risk of neurodegenerative diseases could be monitored for symptom onset to receive early symptomatic treatment or, alternatively, be enrolled in preventive or therapeutic trials. In the future, once treatment options become available that can effectively slow down or halt disease progression, early identification of neurodegenerative disease patients would enable timely initiation of disease-modifying therapies.

Impaired motor function is a hallmark of parkinsonism, and at the same time is increasingly recognized as an important feature of deteriorating brain function in dementia. In community-dwelling elderly, gait abnormalities and parkinsonian signs predict dementia,[4,5] similarly to cognitive tests,[6] and a combination of slow gait and cognitive complaints.[7,8] As a complex trait that requires integration of motor and cognitive skills, loss of manual dexterity embodies the phenotypical

overlap of neurodegenerative diseases.[9-11] We hypothesized that manual dexterity may deteriorate at an early stage of disease and that a simple test of manual dexterity could therefore predict onset of parkinsonism and dementia in the community. We prospectively tested our hypothesis in a large cohort of community-dwelling individuals aged 55 years and older.

METHODS

Study population

The study was embedded in the Rotterdam Study (RS-I), a large, prospective, population-based study in the Netherlands.[12,13] In 1990, inhabitants of the well-defined Ommoord district in the city of Rotterdam who were aged 55 years and older were invited to participate, and 7,983 individuals agreed (first subcohort). In 2000, all inhabitants who had become 55 years of age and older, or moved into the study district since the start of the study were invited to be included in the Rotterdam Study, and 3,011 agreed (second subcohort). The cohort was further extended in 2006 (third subcohort) to a total of 14,926 participants (overall response 72.0 %). By 2012, the first subcohort had a total of five visits (mean interval between visits: five years), whereas the second subcohort had three visits. The third subcohort had not had a follow-up visit yet; therefore, we only included the first two subcohorts for this report.

Manual dexterity assessment was introduced at the fourth follow-up visit for the first subcohort (2002 to 2004; n=2796) and at the first visit for the second subcohort (2000 to 2002; n=2274); we will refer to this assessment as baseline. Participants were extensively screened for parkinsonism and dementia,[14,15] and we

excluded individuals who had parkinsonism or dementia or an unknown status for parkinsonism or dementia at the time of their manual dexterity assessment (n=214). We followed the remaining 4,856 participants until the first of: onset of parkinsonism, onset of dementia, 1 January 2012, or death. At each visit, participants underwent home interviews and medical examinations at the research center. Study follow-up for neurodegenerative diseases was virtually complete.

Assessment of manual dexterity

We used the Purdue Pegboard Test (PPT) to assess manual dexterity.[16] In this test, participants are tasked to place as many cylindrical metal pegs into one of 25 holes in a pegboard as possible in 30 seconds. The test is performed thrice, respectively using left hand, right hand, and both hands simultaneously. The average PPT score is calculated as the sum of each trial divided by 3.

Assessment of covariates

Smoking habits were assessed during home interviews and participants were subsequently categorized as current, former and never smokers. Also, participants were separately asked for parental history of dementia and parkinsonism. Educational level was also assessed and categorized as primary education, lower/intermediate general education or lower vocational education, intermediate vocational education or higher general education, and higher vocational education or university.[17] Subjective memory complaints were assessed using three questions, which could be answered by yes or no. These questions were:

"Do you have more trouble remembering things than before?"; "Does it happen more often that you are on your way to do something and forget what you wanted to do?"; and "Do you more often have trouble finding words during a conversation?" In addition, participants were asked about their preferred hand.

Ascertainment of dementia and Alzheimer's Disease

A detailed description of assessment methods has previously been published.[18] In short, participants were screened for dementia at baseline and follow-up examinations using a three-step protocol,[15] Individuals with a positive screen on either Mental State Examination (MMSE) [19] or the Geriatric Mental Schedule (GMS) organic level [20] were subjected to the Cambridge Examination for Mental Disorders of the Elderly.[21] Additional information was obtained from routinely performed in-person neuropsychological examination, and the total cohort was continuously monitored for dementia through computerized linkage of medical records from general practitioners and the regional institute for outpatient mental healthcare with the study database. Available neuroimaging data were used when required for establishing a diagnosis. For all suspected cases of dementia, a consensus panel led by a consultant neurologist (PJK), decided on the final diagnosis in accordance with standard criteria for dementia (DSM-III-R), AD (NINCDS-ADRDA), and vascular dementia (NINDS-AIREN). Participants were diagnosed with PD dementia if diagnosis of dementia was preceded by a diagnosis of PD at least 1 year prior.

Assessment of parkinsonism and Parkinson's Disease

A detailed description of assessment methods is provided in **Chapter 4.1**. In short, we used four overlapping modalities to screen for potential parkinsonism: in-person examinations (on average every 4 years), in-person interviews, use of antiparkinson medication, and clinical monitoring alerts.

Of all individuals who screened positive in any of these methods, complete medical records (including letters from medical records of specialists and general practitioners) were studied and case reports were drawn up covering all potentially relevant information to establish presence and cause of parkinsonism. These case reports were evaluated by a panel led by an experienced neurologist.

Parkinsonism was defined by presence of hypo- or bradykinesia in combination with ≥1 cardinal sign (resting tremor, rigidity or postural imbalance) observed by any physician, or a clinical diagnosis of parkinsonism by a neurologist or geriatrician (if motor examination details were unavailable). PD was only diagnosed after exclusion of secondary causes, in individuals with at least one of: 1) a clinical PD diagnosis by a neurologist or geriatrician; 2) positive response to dopaminergic treatment. Individuals with parkinsonism who did not fulfill PD criteria were considered secondary parkinsonism cases. After initial diagnosis, medical records of all incident parkinsonism cases continued to be scrutinized until the end of the study period for new information that could lead to a revision of the diagnosis.

Statistical analysis

Analyses included all participants who were free of dementia and parkinsonism at baseline. We used competing risk models to determine the association of PPT

scores with any incident neurodegenerative disease, any dementia, cause-specific dementia (AD and non-AD), any parkinsonism, and cause-specific parkinsonism (PD and non-PD).[22] For any neurodegenerative disease, death was the only competing risk; for any dementia, death and parkinsonism were competing risks (and vice versa for parkinsonism); for cause-specific neurodegenerative diseases, death and other neurodegenerative diseases were competing risks. All analyses were adjusted for age, sex, comprising education, parental history of neurodegenerative diseases, subjective memory complaints, smoking, and MMSE. In order to facilitate clinical interpretation of our results, we report hazard ratios per decreasing point of average Purdue Pegboard Test score.

We performed several sensitivity analyses on the association between manual dexterity and any neurodegenerative disease. First, we investigated the association between average PPT score and any incident neurodegenerative disease after excluding at baseline individuals with any parkinsonian sign (any hypo- or bradykinesia, tremor, cogwheel rigidity, or postural instability) and, separately, excluding individuals with MMSE <26. Second, we consecutively assessed effect modification by age, sex and study subcohort by introducing interaction terms with PPT scores into the main model. After initial analyses showed strong effect modification by age, we stratified further association analyses by age, using the median age as cut-off. Third, we excluded the first 5 years of follow-up to assess whether manual dexterity was associated with long-term risk of neurodegenerative diseases. Fourth, we explored practical implementation of the PPT in clinical practice by using a cut-offs of the average PPT score (-1 standard deviation), which allows for a direct comparison of individuals with low vs. normal/high scores. Fifth, we investigated separate

associations of scores on each PPT task (i.e., left hand, right hand, bimanual) with incident neurodegenerative diseases.

Furthermore, we assessed whether the average PPT score improved prediction of any neurodegenerative disease, dementia, and parkinsonism beyond age, sex, study subcohort, education, smoking, preferred hand, parental history, memory complaints, and MMSE. In line with association analyses that showed strong effect modification by age, we stratified prediction analyses by age, using the median age as cut-off. To express the incremental predictive value of manual dexterity assessment, we used markers of risk classification (continuous net reclassification index [NRI]) and discrimination (integrated discrimination improvement [IDI] and change in concordance statistics [ΔC-statistic]).[23-25]

We lacked information on parental history for 1,121 participants (23%) and for <2% of participants on all other predictors (education [n=45], smoking [n=61], and memory complaints [n=86]. Missing values were handled by multiple imputation using the mean of five imputations, based on all other predictors and the occurrence of incident neurodegenerative disease. Distribution of variables was similar before and after imputation. A (type 1 error) was set at 0.05. Data were handled and analyzed with the IBM SPSS Statistics version 21.0.0.1 (IBM Corp., Somers, NY) and R version 3.2.1.

RESULTS

Baseline characteristics of the study population are shown in *Table 1*. The median age was 70 years (range 55-98), and there were more women than men. Participants in the second subcohort were generally younger than participants in

the first subcohort (*Online Supplementary Table 1*). The distribution of PPT scores was roughly normal (*Online Supplementary Figure 1*). PPT task scores for left, right, and both hands were moderately correlated, with Pearson's correlation coefficients ranging from 0.69 to 0.76 ($p<0.001$ for all pairs), and each task score was highly correlated with the average score ($\rho=0.88$ to $\rho=0.92$, $p<0.001$). For the average PPT scores, one standard deviation below the mean corresponded to 10 pins, whereas two standard deviations below the mean was roughly equivalent to 8 pins.

Table 1 | Study population characteristics.

Characteristic	Population at risk [n=4856]
Age	70.2 [13.9]
Female	2767 [57.8]
Education*	
primary	521 [10.8]
lower	2121 [44.1]
intermediate	1475 [30.7]
higher	694 [14.4]
Smoking	
Never	1474 [30.7]
former	2571 [53.6]
current	750 [15.6]
Parental history of neurodegenerative diseases	1034 [27.7]
≥1 memory complaint	1984 [14.6]
MMSE	28 [2]
≥ 1 Parkinsonian sign	768 [13.3]
Purdue Pegboard Test average task score	11.3 [2.3]

*For continuous characteristics, median [interquartile range] is presented; for categorical characteristics, number [percentage] is presented. MMSE, Mini-Mental State Examination. Parkinsonian signs: hypo- and bradykinesia, tremor, cogwheel rigidity, and postural instability. *Education was categorized as: low = primary only; low-intermediate = lower/intermediate general education or lower vocational education; intermediate-higher = intermediate vocational education or higher general education; high = higher vocational education or university.*

During follow-up (median 9.2 years), 277 participants were diagnosed with a neurodegenerative disease of whom 155 (56%) had AD, 72 (26%) had another primary dementia diagnosis, 33 (12%) had PD, and 17 (6%) had parkinsonism due to other causes. 88% of all incident neurodegenerative disease cases were elderly (i.e., aged >70 years), and the distribution of neurodegenerative disease diagnoses in this group was similar to the overall distribution.(*Online Supplementary Table 3*) Among the middle-aged, the distribution of neurodegenerative disease diagnoses was markedly different: primary parkinsonism diseases made up 42% of all neurodegenerative disease diagnoses, and only 21% of all neurodegenerative disease cases had an AD diagnosis. Lower PPT scores were independently associated with a higher risk of incident dementia and incident parkinsonism, and each decreasing point of average PPT score corresponded to a 28% higher hazard of any neurodegenerative disease.(*Table 2*)

Table 2 | Manual dexterity and the risk of neurodegenerative diseases.

	Any neurodegenerative disease		Dementia		Parkinsonism	
	N cases	HR [95% CI]	N cases	HR [95% CI]	N cases	HR [95% CI]
Overall	277	1.28 [1.18 ;1.41]	227	1.25 [1.14; 1.39]	50	1.41 [1.19; 1.67]
≥70 years	244	1.28 [1.16; 1.41]	209	1.23 [1.12; 1.37]	35	1.39 [1.16; 1.67]
<70 years	33	1.45 [1.18; 1.82]	18	1.54 [1.11; 2.13]	15	1.45 [1.00; 2.08]

HR, hazard ratio per decreasing point of average Purdue Pegboard Test score. 95% CI, 95% confidence interval. Analyses are adjusted for age, sex, study cohort, education, smoking, preferred hand, parental history of neurodegenerative diseases, memory complaints and Mini-Mental State Examination.

Results were similar for Parkinson's Disease (hazard ratio HR=1.35, 95% confidence interval [1.11; 1.67]) and secondary parkinsonism (HR=1.49 [1.12; 1.96]). For dementia, we observed independent associations for Alzheimer's Disease (HR=1.22 [1.06;1.39]) as well as any other type of dementia, and the effect estimate was more distinct for the latter (HR=1.33 [1.14;1.54]).

The association between PPT scores and incident neurodegenerative diseases was stronger in middle-aged participants (age <70 years) than in older participants (p for interaction term <0.001). Furthermore, although we did not observe statistically significant effect modification by sex (p=0.09), the association with any neurodegenerative disease was more pronounced in women.(*Table 3*)

Table 3 | Manual dexterity and the risk of any neurodegenerative disease: sensitivity analyses.

	HR [95% CI]
Average score in total population	1.28 [1.17; 1.40]
in men	1.19 [1.04; 1.36]
in women	1.34 [1.20; 1.51]
subcohort 1	1.33 [1.21; 1.46]
subcohort 2	1.24 [1.01; 1.53]
after exclusion of individuals with MMSE <26	1.22 [1.08; 1.37]
after exclusion of individuals with any parkinsonian sign*	1.30 [1.17; 1.45]
after the first 5 years of follow-up	1.28 [1.16; 1.40]
*Binary cut-off in total population: low** average score*	1.68 [1.24; 2.28]
Left hand task score	1.20 [1.12; 1.28]
Right hand task score	1.20 [1.12; 1.29]
Bimanual task score	1.19 [1.10; 1.29]

*HR, hazard ratio per point of average Purdue Pegboard Test score. 95% CI, 95% confidence interval. Parkinsonian signs: hypo- and bradykinesia, tremor, cogwheel rigidity, and postural instability. MMSE, Mini-Mental State Examination. Analyses are adjusted for age, sex, study cohort, education, smoking, preferred hand, parental history of neurodegenerative diseases, memory complaints and Mini-Mental State Examination. *Average score of < -1 standard deviation, corresponding to an average score of ~10 pins (reference is >-1 standard deviation)*

202 of all incident neurodegenerative disease cases (73%) were participants in the first subcohort. We found no evidence for effect modification by subcohort in the main analysis (p=0.32). The association between average PPT scores and any neurodegenerative disease was not affected by exclusion of individuals with any parkinsonian sign and only mildly by low MMSE scores, and PPT scores remained strongly associated with the incidence of neurodegenerative diseases more than 5 years after PPT assessment. Using a binary cut-off that correspond to an average PPT score of 10 pins, individuals with low scores were at distinctly increased risk of neurodegenerative diseases compared to others.(*Table 3*)

As shown in *Online Supplementary Table 4*, parkinsonism-specific estimates were generally somewhat higher than dementia-specific estimates across several sensitivity analyses. Incorporation of PPT scores significantly improved discrimination (IDI) of any neurodegenerative disease in middle-aged and elderly individuals.(*Table 4*) In addition, there were non-significant improvements in C-statistics and NRI in both age groups. For parkinsonism, IDI improved significantly in both age groups, while NRI improved significantly only in the middle-aged. For dementia, NRI increased significantly in elderly but not in middle-aged, while IDI increased significantly in middle-aged but not in elderly. For all outcomes, C-statistics did not improve significantly after incorporation of PPT scores. C-statistics after introduction of PPT scores were 0.777 [0.736; 0.818] for any neurodegenerative disease, 0.791 [0.752; 0.830] for dementia, and 0.770 [0.682; 0.858] for parkinsonism in the elderly. In the middle-aged, C-statistics were 0.883 [0.816; 0.949], 0.915 [0.846; 0.984], and 0.847 [0.707; 0.986].

Table 4 | Incremental predictive value of manual dexterity assessment for incident neurodegenerative diseases in the community.

Age group		Any neurode-generative disease	Dementia	Parkinsonism
>70 years	NRI [95% CI]	+0.078 [-0.075; +0.215]	+0.017 [0.000; +0.037]	+0.080 [-0.073; +0.220]
	IDI [95% CI]	+0.019 [+0.003; +0.039]	+0.079 [-0.045; +0.220]	+0.016 [+0.002; +0.038]
	ΔC-statistic [95% CI]	+0.009 [-0.005; +0.024]	+0.006 [-0.008; +0.020]	+0.006 [-0.007; +0.018]
≤70 years	NRI [95% CI]	+0.298 [-0.073; +0.466]	+0.012 [-0.007; +0.112]	+0.354 [+0.001; +0.588]
	IDI [95% CI]	+0.072 [+0.017; +0.141]	+0.344 [+0.058; +0.477]	+0.083 [+0.019; +0.212]
	ΔC-statistic [95% CI]	+0.029 [-0.009; +0.067]	+0.011 [-0.011; +0.032]	+0.054 [-0.023; +0.131]

NRI, continuous net reclassification index. IDI, integrated discrimination improvement. The estimates reflect change in prediction of neurodegenerative diseases after addition of average Purdue Pegboard Test scores to a basic model with age, sex, study subcohort, education, smoking, preferred hand, parental history of neurodegenerative diseases, memory complaints, and Mini-Mental State Examination.

DISCUSSION

In this prospective, population-based sample with up to twelve years of follow-up, we found that low scores on a brief and objective test of manual dexterity are associated with incident dementia and parkinsonism beyond age, sex, and common risk factors. This highlights the overlap in the prediagnostic phases of

dementia and parkinsonism diseases, and suggests that manual dexterity testing may contribute to identifying individuals at increased risk of neurodegenerative diseases in the general population.

Before interpreting the results of this study further, a few limitations should be noted. First, our definition of neurodegenerative diseases was limited to dementia and parkinsonism. As a consequence, we did not include a relatively small number of community-dwelling elderly individuals with for instance amyotrophic lateral sclerosis. Second, we lacked histologic confirmation of specific pathologies, which may have led to some misclassification, in particular for disease subtypes such as Lewy-body dementia. In spite of these limitations, our findings add novel insight on the overlap of dementia and parkinsonism diseases. Despite important differences in clinical presentation between AD and PD, patients with AD often show signs of motoric impairment,[26] and cognitive dysfunction and dementia are common in patients with PD.[27] In addition, prospective studies have recently shown that slow gait, in combination with subjective cognitive complaints, is associated with an increased risk of dementia in community residing individuals.[7,8] Manual dexterity requires integration of both cognitive and motor skills,[9-11] and patients with cognitive dysfunction are generally impaired in fine and complex hand motor activity compared with healthy elderly individuals.[28] While a previous study showed that a combination of manual dexterity, dynamometry and a neurological examination prospectively predicted worsening MMSE scores in community-dwelling elderly,[29] manual dexterity has, to the best of our knowledge, not previously been studied prospectively for its association with incident dementia and parkinsonism in a community-based sample. In patient population samples, manual dexterity predicted onset of dementia in PD patients,[10,30] but did not predict conversion to AD in MCI patients.[31]

We hypothesized that mild impairment across a variable combination of cognitive and motoric functions may lead to a moderate decline in manual dexterity in individuals with a prediagnostic neurodegenerative disease. We observed that dexterity is strongly associated with the risk of both dementia and parkinsonism, which further supports phenotypical overlap between neurodegenerative diseases and highlights the involvement of motor function in dementia and AD. Whether this overlap reflects shared pathophysiological mechanisms remains subject of debate. While there is pathologic overlap between AD and PD,[32] there are important differences in traditional risk factor profiles, including discordant prospective associations of smoking and serum uric acid with AD and PD.[33-36] In addition, only relatively little genetic overlap between AD and PD has been identified to date,[37] suggesting that various traditional and genetic risk factors may lead to similar pathology and symptomatology via different pathways.[38] Therefore, we hypothesize that low PPT scores may be a consequence of either synucleinopathy, tauopathy, amyloidosis or vascular lesions, and there may be overlap of these pathologies in at least a subgroup of clinical neurodegenerative disease patients. Further research is warranted to disentangle the genetic, pathological and clinical overlap in cognitive and motoric impairment, and their progression to neurodegenerative diseases.

We observed that the overall association of manual dexterity with incident neurodegenerative diseases was somewhat stronger in middle-aged individuals than in elderly. This may be explained by a different distribution of neurodegenerative disease diagnoses in the former, especially the low proportion of AD cases, as well as by a lower prevalence of mild non-neurodegenerative disability that could influence PPT scores independently of brain function, such as locomotor diseases. Still, we note that the number of cases among middle-aged

participants was small. Furthermore, while we observed that PPT scores were distinctly associated with incident neurodegenerative diseases independent of traditional risk factors, improvement in markers of prediction were generally relatively small and some of these changes were non-significant. In addition, while we assessed the predictive value of PPT scores over subjective memory complaints and MMSE scores, there are several more detailed cognitive and motoric assessments which have higher predictive value for dementia.[6] Also, since we did not perform baseline gait speed assessments, we could unfortunately not assess the incremental predictive value of PPT scores for neurodegenerative diseases over gait speed. In a previous study, individuals with abnormal baseline gait had worse manual dexterity.[4] Future studies that prospectively investigate gait speed and manual dexterity as risk factors for dementia in one population can formally compare their predictive value, and may unravel whether gait speed and manual dexterity reflect the same pathological process or different pathological processes in prediagnostic dementia.

As of yet, no disease-modifying treatments are available for dementia, which may in part be due to diagnosis at advanced disease stages. The need to assess treatment efficacy at an early, pre-symptomatic stage of disease has led to discussion about (genetic) screening of asymptomatic participants for neuroprotective trials. Trials have recently been designed for individuals with rare, highly risk-increasing traits for AD and PD, both genetic[39] and non-genetic (e.g., REM-sleep behavior disorder[40]). Although these trials are important to determine the modifiability of disease progression in these subgroups of high-risk individuals, a large proportion of prediagnostic AD and PD patients do not possess these rare traits.[41-43] As a consequence, results from these trials may not be applicable to the majority of patients with neurodegenerative disease.

Therefore, it will be vital to have effective community-wide screening programs for neurodegenerative diseases that identify the majority of future clinical neurodegenerative disease patients at an early stage.

In our population-based sample, AD and PD made up 56% and 12% of all incident neurodegenerative disease diagnoses, respectively, while 32% had a different dementia or parkinsonism diagnosis. Therefore, combined screening for dementia and parkinsonism, rather than separate screening algorithms for AD and PD, may not only be time-saving, but also allow detection of one third of future neurodegenerative disease patients who might otherwise be missed. As a first step to identify individuals at increased risk of neurodegenerative diseases, community-wide screening programs may comprise basic demographics, a short interview for common risk factors and rapid, consulting room tests such as gait speed and manual dexterity assessment. Individuals at risk of AD, PD and other neurodegenerative diseases could be referred to geriatric or neurologic clinics for refined risk stratification, and possibly for inclusion in neuroprotective trials. Our findings suggest that manual dexterity testing might be part of a such a joint screening algorithm.

In conclusion, this study demonstrates that a simple, non-laboratory test of manual dexterity can help to predict neurodegenerative diseases in the community. This might contribute to identifying people in the community for refined screening and selection of individuals most suitable for neuroprotective intervention trials.

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Chapter 3.3

Gait, cognitive decline and dementia

Chapter 3.3

ABSTRACT

Poor gait has recently emerged as a potential prodromal feature of cognitive decline and dementia. We assessed to what extent various aspects of poor gait are independently associated with cognitive decline and incident dementia. We leveraged detailed quantitative gait (GAITRite™) and cognitive assessments in 4,258 dementia-free participants (median age 67 years, 55% women) of the population-based Rotterdam Study (baseline 2009-2013). We summarized 30 gait parameters into seven mutually independent gait domains and a Global Gait score. Participants underwent follow-up cognitive assessments between 2014-2016 and were followed for incident dementia until 2016 (median 4 years). We observed that three independent gait domains (Base of Support, Pace and Rhythm) and Global Gait were associated with cognitive decline. Two independent gait domains (Pace and Variability) and Global Gait were associated with incident dementia. Associations of gait with cognitive decline and incident dementia were only present in individuals who had been cognitively unimpaired at baseline. In conclusion, poor performance on several independent gait domains precedes cognitive decline and incident dementia.

BACKGROUND

Poor gait has recently emerged as a potential prodromal feature of cognitive decline and dementia.[1-3] However, it is unclear to what extent various aspects of poor gait independently associate with cognitive decline and incident dementia. Gait encompasses a broad array of quantifiable parameters, such as speed, stride width or stride time. Although these parameters are to a varying extent correlated, they reflect various aspects of gait that can be summarized into mutually independent gait domains, such as Pace ([which includes several parameters, including:] gait speed), Base of Support (stride width), Rhythm (stride time) or Variability (variability in stride time and width).[4]

Interestingly, several independent gait domains have been cross-sectionally associated with cognitive functioning.[5] Also, in the Mayo Clinic Study of Aging several gait parameters were associated with decline in global and domain-specific cognitive functioning.[6] However, only one relatively small (n=427) population-based study has published data on associations of independent gait domains with cognitive decline and dementia. In that study, worse Pace was associated with a decline in Global Cognition over a median 2-year follow-up period, while worse Variability and Rhythm were associated with incident dementia.[7] The findings of that study warrant corroboration in a larger sample with longer follow-up. They also leave the important question unanswered whether associations of poor gait with cognitive decline and incident dementia vary by baseline cognitive functioning. Of particular interest is whether poor gait may be a determinant of cognitive decline and incident dementia in cognitively unimpaired individuals.

We hypothesized that several gait domains are independently associated with cognitive decline and incident dementia. We also hypothesized that associations of poor gait with cognitive decline and incident dementia would remain present in individuals free of cognitive dysfunction at baseline. We tested these hypotheses by leveraging detailed quantitative gait assessments, serial cognitive assessments, and follow-up for incident dementia in a large, population-based cohort.

METHODS

Study characteristics

The study was embedded in the Rotterdam Study, a large, prospective, population-based study in the Netherlands.[8,9] In 1990, inhabitants of the well-defined Ommoord district in the city of Rotterdam who were aged 55 years and older were invited to participate, and 7,983 individuals agreed (first subcohort). In 2000, all inhabitants who had become 55 years of age and older or who moved into the study district since the start of the study were invited to be included in the Rotterdam Study, and 3,011 agreed (second subcohort). The cohort was further extended in 2006 (third subcohort; age range 45 years and older) to a total of 14,926 participants (overall response 72%). Participants were subsequently invited for follow-up examinations at the research center, with a mean interval between visits of four years. By 2016, the first subcohort had a total of up to six visits, whereas the second subcohort had four visits, and the third subcohort had two visits.

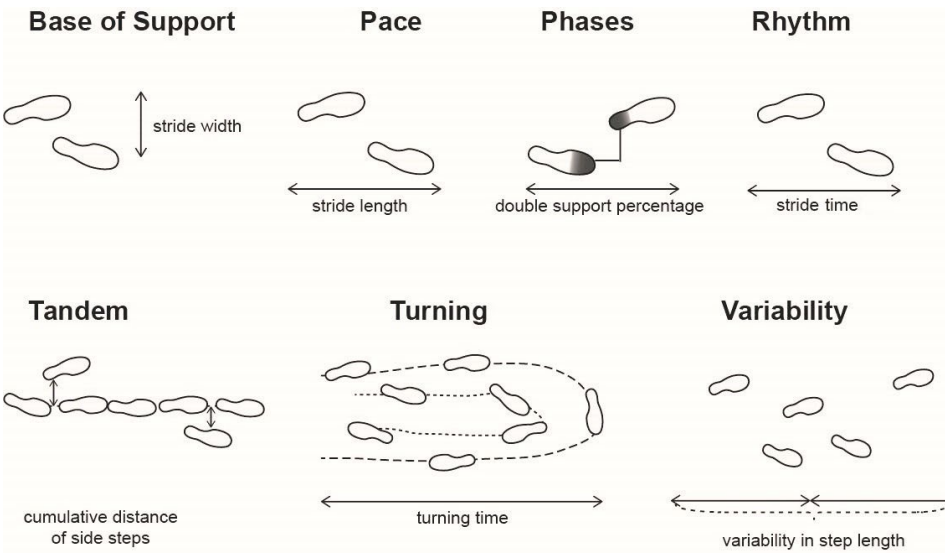
Gait assessments were implemented into the core protocol of the Rotterdam Study in 2009. Between 2009 and 2013, 4,258 participants free of dementia across the three subcohorts underwent detailed gait and cognitive assessments. We will refer to this assessment as "baseline". Between 2014 and 2016, 3,253 (76%) of these participants underwent follow-up cognitive assessments. Reasons for missing data on a follow-up cognitive assessment were death (n=208), follow-up cognitive assessment planned after current study period (n=167), or refusal or inability (n=697). The follow-up period for dementia was defined as the interval between baseline dementia screening at the research center and the first of: diagnosis of dementia, death or January 1, 2016. Follow-up for dementia included in-person examinations as well as continuous surveillance through electronic linkage of the study database with medical records, and was 99% complete.[10]

Assessment of gait

Gait was evaluated using a 5.79-m long walkway (GAITRite™ Platinum; CIR systems, Sparta, NJ: 4.88-m active area; 120-Hz sampling rate). The reliability and validity of this device have been previously established.[5,11-13] The standardized gait protocol comprises three walking conditions: normal, turning and tandem walk. In the normal walk, which was repeated up to eight times, participants walked at their usual pace across the walkway. We calculated mean values across these walks, apart from the first walk, which we considered a practice walk. In turning, participants walked at their usual pace, turned halfway, and returned to the starting position. In the tandem walk, participants walked heel-to-toe on a line across the walkway. Based on the recorded footfalls, the walkway software calculated 30 parameters, including 25 from the normal walk, 2 from turning and

3 from the tandem walk.(*Figure 1*) We have previously provided a description of these parameters (**Chapter 2.1**). All recordings were visually inspected.

Figure 1 | Independent gait domains.



To summarize gait parameters into independent domains, we performed a principal component analysis. This yielded 7 independent gait domains: Base of Support, Pace, Phases, Rhythm, Tandem, Turning and Variability. For each gait domain, a single gait parameter that has high correlation with the domain is illustrated.

From a clinical point of view, an individual with "poor" gait (i.e., z-score=2 or $\geq$1 double step during tandem walk) may have a combination of some of the following gait characteristics: low cadence (<91 steps/min), highly-variable step length (average standard deviation in step length>5 cm), high double support time (>0.4 s), low gait speed (<81 cm/s), difficulty maintaining balance while tandem walking ($\geq$1 double step), slow turning (>4 s), or wide base (>18cm).

Assessment of cognitive functioning and manual dexterity

We previously published a detailed description of our assessment methods of cognitive functioning and manual dexterity.[14] We used the Stroop color word test,[15] Letter-Digit Substitution Test (LDST),[16] Word Fluency Test,[17] 15-Word List Learning Test (WLT)[18] and the Purdue Pegboard Test (PPT).[19]

The abbreviated Stroop test consists of three subtasks in which the participant is shown a colored card with 40 items that have to be named.[15] In naming task, the participants are asked to name the printed words (primary latent domain: *speed of reading*); in the color task the participants are asked to name the printed colors (*speed of color naming*); in the interference task the participants are asked to name the color in which each color-name is printed (*information processing on an interference task*). For each trial, the time to complete the task was used as the outcome. We inverted Stroop test scores to facilitate a consistent interpretation of scores across cognitive tests, i.e. that a higher score indicates better cognitive functioning. The LDST is a modified version of the Symbol Digit Modalities Test[16] and asks the participants to make as many letter-digit combinations as possible in 60 seconds, following an example that shows correct combinations (*information processing speed / executive function*). In the Word Fluency Test, participants were asked to name as many animals as possible within 60 seconds (*semantic fluency*).[17] For both the Word Fluency Test and LDST the number of correct answers was used as the outcome. The WLT comprised of three tasks: immediate recall, delayed recall and recognition. For immediate recall, participants were presented three times with a sequence of 15 words and subsequently asked to recall as many of these words as possible (*verbal learning*). Free delayed recall was tested approximately 10 minutes later (*retrieval from verbal memory*). Recognition was tested by presenting the participants a sequence of 45 words, the 15 words

presented during the Immediate recall mixed with 30 new words. Participants were asked whether they recognized the words as the ones presented to them during the immediate recall trial (*recognition from verbal memory*). Outcome variables were the mean of the number of words recalled over the first three trials (as a summary score for immediate recall), the number of words remembered after the 10-minute delay (as a score for free delayed recall) and the number of correctly recognized words during the recognition trial (as a score for recognition).

In the PPT, participants are asked to place as many cylindrical metal pegs into 1 of 25 holes in a pegboard as possible in 30 seconds. The test is performed three times, one each using the left hand, right hand, and then both hands simultaneously. In analyses, we used the mean PPT score which is the sum of each trial divided by three.

Assessment of dementia

A detailed description of assessment methods has previously been published.[20] In short, participants were screened for dementia at baseline and subsequent center visits with the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Those with a MMSE score <26 or GMS score >0 underwent further investigation and informant interview, including the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX). In addition, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study database with medical records from general practitioners and the regional institute for outpatient mental health care. This provided detailed information and was used for diagnosis of dementia and for accurately

determining time of diagnosis. Available information on clinical neuroimaging was used if required for diagnosis of dementia subtype.

A consensus panel led by a consultant neurologist established the final diagnosis according to standard criteria for dementia (DSM-III-R) and Alzheimer's Disease (NINCDS–ADRDA).

Statistical analysis

Gait parameters with a skewed distribution were log-transformed, and all continuous gait parameters were standardized. To summarize gait parameters into independent domains, we performed a principal component analysis (PCA) with Varimax rotation, as previously described in detail.[4] This yielded 7 gait domains with an eigenvalue > 1, which we labeled in accordance with the gait parameters that were highly correlated with that domain: Base of Support, Pace, Phases, Rhythm, Tandem, Turning and Variability.[4] Illustrations of gait domains and an overview of highly correlated parameters per domain is presented in **Chapter 2.1**. Global Gait was calculated by averaging the normal walk gait domains into a standardized Z-score.[4] Baseline Global Gait explained 87% of the variance in baseline gait parameter values, and follow-up Global Gait explained 87% of the variance in follow-up gait parameter values.

We calculated Global Cognition (g-factor) as the first component of an unrotated PCA that incorporates tasks from all available cognitive functioning tests. Although Stroop and WLT comprised several tasks, we only used data from the most complicated task of each (i.e., the interference task for Stroop and the 15-minute delayed recall task for WLT) in calculating the g-factor to prevent highly

correlated tasks distorting factor loadings in the PCA. Contrary to our previous work,[14] we did not include the PPT in the calculation, since it is strongly influenced by motor function and was previously already shown to predict incident dementia in this population.[19] Baseline Global Cognition explained 54% of the variance in baseline cognitive test scores, and follow-up Global Cognition score explained 57% of the variance in follow-up cognitive test scores.

All individuals who participated in gait assessments had analyzable data on the normal walk, but 219 (5%) individuals did not perform the tandem walk and 162 (4%) did not perform the turning walk. 427 (10%) study participants did not complete one or two cognitive tasks. In total, 436 (10%) individuals had missing data on the tandem walk, turning walk or one or two cognitive tasks. We imputed missing data on the turning and tandem walk based on age, sex, normal walk gait parameters, follow-up time, and incident dementia status. We imputed cognitive task values using based on age, sex, educational attainment, other cognitive test scores, follow-up time, and incident dementia status. The distribution of gait and cognitive test scores before and after imputation was similar.

We conducted our analyses in three blocks. First, we assessed the association between baseline Global Gait and Global Cognition using linear regression models with adjustment for age, sex, education and time-interval between assessments. We modeled change in cognition by using the follow-up value of the cognitive outcome as dependent variable while adjusting for its baseline value. This approach has shown to yield more statistical power than calculating change as difference in baseline and follow-up values.[21] In the analysis on Global Cognition, we adjusted the follow-up Global Cognition score for baseline Global Cognition score; in the analyses on each individual test score, we adjusted for the baseline test score. We repeated these analyses to assess the association between

separate gait domains or original gait parameters and change in Global Cognition. In addition, we repeated these analyses to assess changes in separate cognitive test scores instead of Global Cognition. We also performed a sensitivity analysis in which we additionally adjusted the association between Global Gait and longitudinal change in the Stroop interference task for Stroop naming and color task test scores, to assess whether baseline gait was independently associated with longitudinal decline in information processing on an interference task.

Second, we assessed the association between baseline quantitative gait parameters and incident dementia using Cox proportional hazards models with adjustment for age, sex and education. In sensitivity analyses, we repeated the dementia analysis after the following exclusions: the first year of follow-up, individuals with a history of stroke, or individuals with prevalent parkinsonism. Furthermore, we repeated the dementia analysis after additional adjustment for the baseline PPT score or for the baseline Global Cognition score. Separately, we restricted the outcome to Alzheimer's Disease and non-Alzheimer's Disease dementia, respectively.

Third, we assessed effect modification of the associations of Global Gait with decline in Global Cognition and incident all-cause dementia by age, sex or Global Cognition by introducing multiplicative interaction terms of these variables with Global Gait in separate models, and subsequently stratified by cognitive dysfunction status (defined as age-, sex- and education-adjusted Global Cognition z-score < -1).

Data were handled and analyzed with the IBM SPSS Statistics version 23.0.0.0 (IBM Corp., Somers, NY) and R version 3.2.4. We adjusted the statistical

significance threshold for multiple hypothesis testing of correlated variables.[22] For associations of original gait parameters with decline in Global Cognition and incident dementia the adjusted threshold was p=0.003. For associations of independent gait domains and decline in cognitive tests the adjusted threshold was p=0.004. Regression coefficients and hazard ratios are presented per standard deviation of "worse" gait.

In sensitivity analyses, we assessed whether cognitive decline and the risk of incident dementia was different in individuals with complete data vs. in individuals who did not complete the tandem walk, turning walk or one or two cognitive tasks, adjusting for age, sex and educational attainment. In post-hoc exploratory analyses, we also assessed whether baseline cognitive functioning was associated with longitudinal change in gait.

RESULTS

The average age in the study population at baseline was 67 years, 55% of study participants were women, and just over half of the study population attained a higher vocational or university education.(*Table 1*)

The average age was somewhat lower in the subgroup with two cognitive assessments, while the proportion with higher vocational or university education and baseline Global Cognition and Global Gait scores were somewhat higher than in the total study population.(*Table 1*) Compared to individuals with complete data on all walks, individuals who did not complete baseline tandem walk, turning walk or one or two cognitive tasks were generally older (mean age 75.7 vs. 66.3

years), more commonly female (60.5% vs. 54.7%), and less commonly highly-educated (44.1% vs. 55.6%).

Table 1 | Population characteristics.

Characteristic	Population	
	Dementia analysis* [N=4,258]	**Cognitive decline analysis** [N=3,253]**
Age, years, mean [SD]	67 [9]	66 [9]
Women, N [%]	2395 [55]	1820 [56]
Higher vocational or university education, N [%]	2358 [54]	1837 [56]
Baseline Global Cognition, mean [SD]	0.0 [1.0]	+0.2 [0.9]
Baseline Global Gait, mean [SD]	0.0 [1.0]	+0.1 [0.9]

N, number. SD, standard deviation.
For Global Cognition and Global Gait, higher values represent better performance.
**Dementia follow-up comprised both in-person examinations at the research center as well as continuous surveillance for dementia through electronic linkage of the study database with medical records from general practitioners and the regional institute for outpatient mental health care.*
***The subgroup with serial cognitive assessments at the research center. Reasons for missing data on a follow-up cognitive assessment were death (n=208), follow-up cognitive assessment planned after current study period (n=167), or refusal or inability (n=697).*

Baseline gait and cognitive decline

3,253 participants underwent follow-up cognitive assessments after a median interval (between cognitive assessments) of 5 years. Of all 30 measured original gait parameters, 20 were nominally associated with decline in Global Cognition, including 13 that survived multiple hypothesis testing.(*Online Supplementary material 3*)

Of the seven independent gait domains, Pace ([regression coefficient standardized by baseline gait and cognitive scores] β=0.06; 95% confidence

interval [0.04;0.09];p[-value] <0.001), Base of Support (β=0.03 [0.01;0.05];p=0.003) and Rhythm (β=0.02 [0.00;0.04];p=0.02) were associated with decline in Global Cognition.(*Figure 2*)

Pace was associated with a decline in each cognitive test except the Word Learning Test recognition task, and Pace was most distinctly associated with decline in the Word Fluency Test (β=0.09 [0.06;0.11];p<0.001) and Word Learning Test immediate recall task (β=0.09 [0.06;0.11];p<0.001). Base of Support was associated with decline in the Stroop interference (β=0.05 [0.02;0.07];p<0.001) and naming task (β=0.03;[0.00;0.05];p=0.03). Rhythm was associated with decline in the Stroop interference task (β=0.03;[0.00;0.05];p=0.04) and Word Fluency Test (β=0.03;[0.01;0.06];p=0.01). Variability was associated with decline in the Stroop naming (β=0.03;[0.00;0.05];p=0.02), color (β=0.04 [0.02;0.06];p<0.001), and interference (β=0.03;[0.00;0.05];p=0.03) tasks.

Global Gait was also associated with subsequent decline in Global Cognition (β=0.06 [0.03;0.08];p<0.001). Baseline Global Gait was statistically significantly associated with decline in each cognitive test apart from the Word Learning Test delayed recall task, and the most distinct effect estimate was for the association with decline in Stroop interference task score (β=0.09;[0.06;0.12];p<0.001).(*Table 2*) After additional adjustment of the association between Global Gait and longitudinal change in the Stroop interference task for Stroop naming and color task test scores, the association only marginally attenuated (β=0.08 [0.05;0.10];p<0.001)."

Baseline gait and incident dementia

During follow-up (median 4 years;range 1-6 years), 78 individuals were diagnosed with incident dementia, including 64 (82%) with Alzheimer's Disease. Twenty-three original gait parameters were nominally associated with incident dementia; of these, 4 associations survived the multiple hypothesis-adjusted statistical significance threshold,(*Online Supplementary material 3*) including gait speed (HR=1.49 [1.19;1.86];[p=0.001]). Of the independent gait domains, Pace (hazard ratio [HR]=1.33 [1.04;1.71];p=0.02) and Variability (HR=1.26 [1.01;1.56];p=0.04) were associated with incident dementia. We also observed a suggestive, albeit not statistically significant association of Phases with incident dementia (HR=1.21 [0.97;1.51];p=0.09).(*Figure 2*) One standard deviation decrease in Global Gait was associated with an increased hazard of dementia (HR=1.29 [1.08;1.54];p=0.006).

Effect modification by baseline cognitive functioning

The association between Global Gait and decline in Global Cognition varied substantially by baseline cognitive functioning (p for interaction term=0.04). In analyses stratified by baseline cognitive dysfunction, the association of Global Gait with decline in Global Cognition was apparent in individuals without baseline cognitive dysfunction (β=0.05 [0.02;0.07];p<0.001) but not in individuals with baseline cognitive dysfunction (β=0.03 [-0.03;0.09];p=0.38). We observed suggestive, yet not statistically significant effect modification by sex of the association between Global Gait and decline in Global Cognition (p=0.06), with a higher effect estimate in men (β=0.10 [0.07;0.13];p<0.001) than in women (β=0.03 [0.01;0.06];p=0.02). We did not observe evidence for effect modification of the association between Global Gait and decline in Global Cognition by age (p=0.37).

Table 2 (part I) | Baseline gait domains: associations with subsequent decline in cognitive test score.

		Baseline gait domain							
		Base of Support	**Pace**	**Phases**	**Rhythm**	**Tandem**	**Turning**	**Variabi-lity**	***Global Gait***
Decline in cognitive test score	**Letter-Digit Substitution**	0.02 [0.00; 0.04]	0.04 [0.02; 0.06]	0.01 [-0.01; 0.03]	0.02 [-0.01; 0.04]	0.01 [-0.01; 0.03]	0.00 [-0.02; 0.02]	0.02 [0.00; 0.03]	0.05 [0.03; 0.07]
	Stroop naming task	0.03 [0.00; 0.05]	0.08 [0.05; 0.11]	-0.01 [-0.04; 0.01]	0.02 [-0.01; 0.05]	0.01 [-0.02; 0.03]	0.01 [-0.01; 0.04]	0.03 [0.00; 0.05]	0.07 [0.04; 0.10]
	Stroop color task	0.01 [-0.01; 0.03]	0.06 [0.03; 0.08]	0.00 [-0.02; 0.02]	0.01 [-0.02; 0.03]	-0.01 [-0.03; 0.01]	-0.02 [-0.04; 0.01]	0.04 [0.02; 0.06]	0.04 [0.01; 0.06]
	Stroop interference task	0.05 [0.02; 0.07]	0.08 [0.06; 0.11]	0.00 [-0.02; 0.03]	0.03 [0.00; 0.05]	0.01 [-0.02; 0.03]	0.02 [0.00; 0.05]	0.03 [0.00; 0.05]	0.09 [0.06; 0.12]
	Word Fluency	0.02 [0.00; 0.05]	0.09 [0.06; 0.11]	0.00 [-0.02; 0.02]	0.03 [0.01; 0.06]	0.00 [-0.03; 0.02]	-0.01 [-0.03; 0.02]	0.01 [-0.02; 0.03]	0.05 [0.02; 0.08]

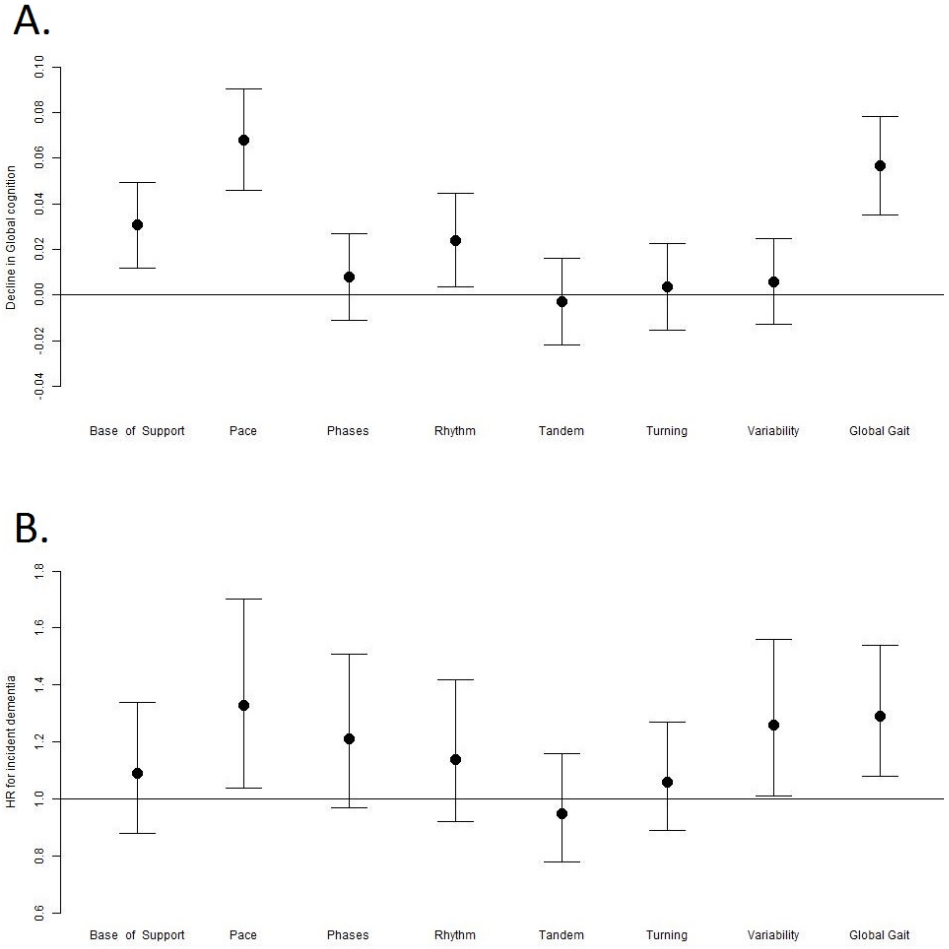
Table 2 (part II) | Baseline gait domains: associations with subsequent decline in cognitive test score.

		Baseline gait domain							
		Base of Support	**Pace**	**Phases**	**Rhythm**	**Tandem**	**Turning**	**Variabi-lity**	***Global Gait***
Decline in cognitive test score	**Word Learning - delayed recall task**	0.01 [-0.02; 0.04]	0.05 [0.02; 0.08]	-0.02 [-0.04; 0.01]	0.02 [-0.01; 0.05]	0.00 [-0.03; 0.02]	-0.01 [-0.03; 0.02]	0.01 [-0.02; 0.04]	0.03 [-0.01; 0.06]
	Word Learning - immediate recall task	0.03 [0.00; 0.05]	0.09 [0.06; 0.13]	0.00 [-0.03; 0.03]	0.03 [0.00; 0.06]	0.00 [-0.03; 0.03]	0.01 [-0.02; 0.04]	-0.01 [-0.03; 0.02]	0.06 [0.03; 0.09]
	Word Learning - recognition task	0.02 [-0.01; 0.05]	0.03 [-0.01; 0.06]	0.00 [-0.03; 0.03]	0.03 [0.00; 0.06]	0.00 [-0.03; 0.03]	0.00 [-0.03; 0.03]	0.02 [-0.01; 0.05]	0.04 [0.01; 0.07]
	Global Cognition	0.02 [0.00; 0.04]	0.04 [0.02; 0.06]	0.01 [-0.01; 0.03]	0.02 [-0.01; 0.04]	0.01 [-0.01; 0.03]	0.00 [-0.02; 0.02]	0.02 [0.00; 0.03]	0.05 [0.03; 0.07]

The presented values are regression coefficients of the association between gait domains and change in cognitive functioning, standardized by baseline gait and cognitive scores. We modeled change by using the follow-up value of the cognitive outcome as dependent variable while adjusting for its baseline value. Positive correlation coefficients indicate that poor baseline gait correlated with decline in cognitive functioning. We inverted Stroop test scores to facilitate a consistent interpretation of scores across cognitive tests, i.e. that a higher score indicates better cognitive functioning. Multiple hypothesis-adjusted statistical significance threshold was set to p=0.004. Color indicates p-value of the association:

<0.001	<0.004	<0.01	<0.05	≥0.05

Figure 1 | Baseline gait domains: associations with subsequent decline in Global Cognition and incident dementia.



A. Association of baseline independent gait domains with subsequent decline in Global Cognition. For all gait domains, higher scores correspond with worse gait. Dots represent regression coefficients standardized by baseline gait and cognitive scores, bars indicate 95% confidence intervals. Regression coefficients were standardized by baseline gait and cognitive scores. Analyses were adjusted for age, sex and education.
B. Association of independent gait domains with incident dementia. For all gait domains, higher scores correspond with worse gait. HR, hazard ratio per standard deviation "worse" gait. Dots represent hazard ratio, bars represent 95% confidence interval. Analyses were adjusted for age, sex and education.

In line with the present effect modification on the association between Global Gait and Global Cognition, the association between Global Gait and incident dementia also varied substantially by baseline cognitive functioning (p for interaction term=0.008). In analyses stratified by baseline cognitive dysfunction, we only observed an association of Global Gait with incident dementia in individuals without baseline cognitive dysfunction (HR=1.28 [0.96;1.69];p=0.09), which was not apparent in individuals with baseline cognitive dysfunction (HR =1.03 [0.80;1.33];p=0.82). We observed no statistically significant effect modification of the association between Global Gait and incident dementia by age (p=0.44) or sex (p=0.46).

Sensitivity analyses and post-hoc analyses

The association between Global Gait and incident dementia remained robust after exclusion of the first year of follow-up (HR=1.28 [1.06;1.54];p=0.01), among individuals without a history of stroke (HR=1.31 [1.10;1.57];p=0.002), in those without prevalent parkinsonism (HR=1.33 [1.10;1.62];p=0.004), or after additional adjustment for Purdue Pegboard score (HR=1.26 [1.05;1.51];p=0.02). The hazard ratio of Global Gait for incident non-Alzheimer's Disease dementia (HR=1.66 [1.13;2.45];p=0.01) was higher than for incident Alzheimer's Disease dementia (HR=1.22 [0.99;1.49];p=0.06). The association between Global Gait and incident dementia attenuated and was no longer statistically significant after additional adjustment for baseline Global Cognition (HR=1.16 [0.96;1.40];p=0.12). Compared to individuals who completed all walks, individuals who did not complete the baseline tandem walk, turning walk or one or two cognitive tasks generally had more distinct cognitive decline at the follow-up assessment (β=0.03

[0.01;0.05];p=0.006) and an increased risk of incident dementia (HR=2.98 [1.82;4.85];p<0.001).

We had follow-up gait assessment data on 1,701 of 4,258 participants (39.9%). In this subgroup, baseline Global Cognition was associated with longitudinal decline in Global Gait (β=0.09 [0.04;0.14];p=0.001). Baseline Stroop (each task), Word Fluency Test and LDST scores were also associated with longitudinal decline in Global Gait.(*Online Supplementary material 4*)

DISCUSSION

In this large, population-based study, worse quantitative gait was strongly associated with subsequent decline in cognitive functioning and the risk of dementia. After stratifying by baseline cognitive functioning, these associations were only present in cognitively unimpaired individuals. We identified independent associations of several gait domains with cognitive decline and the risk of dementia, suggesting that a detailed assessment of gait can potentially provide novel insight into the etiology of cognitive decline and dementia. From a clinical perspective, associations of poor gait with decline in specific cognitive functions may also have predictive utility.

After adjustment for multiple-testing, 13 gait parameters were associated with cognitive decline and 4 gait parameters with incident dementia. Since some of these parameters are strongly correlated (e.g., step time and stride time), we aimed to unravel associations of underlying, independent gait domains with cognitive decline and incident dementia. This approach is similar to the approach used in a British population-based study and the Einstein Ageing Study.[7,23] In

both studies as well the Rotterdam Study, the following independent domains were identified: Pace, Rhythm and Variability. The Base of Support domain in Rotterdam Study and the Postural Control domain in the British study both included step width but had a different contributing parameter (step width variability vs. step length asymmetry). Furthermore, we identified Phases as an independent domain, and our assessment of gait under tandem and turning conditions facilitated the identification of additional parameters that contributed to two more domains (which we named Tandem and Turning). We note that the British study also systematically collected data on left-right differences, which facilitated the identification of the Asymmetry domain. The Einstein Ageing Study is the only previous study that we are aware of to have also reported associations of independent, quantitative gait domains with cognitive decline as well as incident dementia. That study had a 10-fold smaller sample size than this study (in the dementia analysis: 4,258 vs. 399 individuals) and only half the follow-up duration (5 vs. 2 years). These differences likely contributed to the identification of a larger number of independent gait domains in the Rotterdam Study (7 vs. 3 domains), additional associations of gait domains with decline in global and domain-specific cognitive functioning as well as incident dementia, and subgroup differences by baseline cognitive functioning. In both the Einstein Ageing Study and the Rotterdam Study, worse Pace was associated with decline in Global Cognition, and the domains Base of Support and Rhythm were each also independently associated with decline in Global Cognition in the Rotterdam Study. Furthermore, several of these gait domains were associated with decline in specific cognitive functions in the Rotterdam Study, including executive functioning, memory, semantic fluency, and information processing on an interference task. These observations may have predictive utility, for instance, individuals with poor Pace and Base of Support may be at increased risk of

impairment in the ability to process interfering information. Worse Variability was associated with incident dementia in both the Einstein Ageing Study and the Rotterdam Study. In the Einstein Ageing Study, the association of Rhythm with incident dementia was statistically significant while the association of Pace was not, while the association with incident dementia of Pace but not of Rhythm was statistically significant in the Rotterdam Study. We note that hazard ratios for both domains were direction-consistent across both studies.

Importantly, after stratification by baseline cognitive functioning, the associations of poor gait with cognitive decline and incident dementia in the Rotterdam Study were only present in individuals who did not have objective cognitive dysfunction at the time of gait assessment. This observation suggests that cognitively unimpaired individuals with poor performance on specific gait domains (Variability and Pace) may constitute a currently under-recognized group at higher risk of dementia. It also suggests that decline in independent aspects of gait may precede decline in cognitive abilities and functional independence in some of these individuals. Previous studies have shown that longitudinal decline of gait speed is associated with incident dementia, even after accounting for low baseline gait speed.[24,25] Traditionally, damage to specific brain regions in specific subtypes of dementia diseases was believed to be associated with poor performance on particular gait domains, for instance, basal ganglia pathology with tendency to shuffle [Phases] in Parkinson's Disease Dementia, or cerebellar pathology for poor heel-to-toe balance [Tandem] in Multiple System Atrophy-C. However, there is now a growing understanding that widespread pathology to the cerebral cortex may contribute to gait decline among patients with Alzheimer's Disease or vascular dementia.[7,26] Furthermore, several cross-sectional studies in individuals (still) free of dementia suggest that the regional distribution

of amyloid-β (Aβ) deposition is associated with specific gait parameters.[27,28] Furthermore, higher cerebral Aβ deposition is associated with subsequent decline in several gait parameters.[29] Also, the association between cerebral Aβ deposition with slow gait speed may be more distinct in individuals with mild cognitive impairment than in individuals who are cognitively unimpaired.[30] Furthermore, widespread disruption of microstructural white matter integrity may contribute to poor gait.[31,32] Interestingly, microstructural integrity and comorbidities may moderate effects of white matter hyperintensities on gait, as previous studies showed that white matter hyperintensities were more distinctly associated with gait speed in individuals with impaired microstructural integrity or with other conditions that affect gait (e.g., poor vision, low forced vital capacity).[33,34] In the coming years, prospective cohort studies will accrue sufficient follow-up for dementia to robustly quantify how much damage in each of these (micro-)structures explains the association between gait and incident dementia. It is also noteworthy that previous studies have shown that the relationship between longitudinal decline in gait and cognition in the ageing population might be bidirectional.[35-38] In the Mayo Clinic Study of Aging, baseline gait speed was inversely associated with subsequent cognitive decline while baseline cognition was not associated with subsequent decline in gait speed, yet, we note that no other aspects of gait were examined.[35] In this study, we observed in post-hoc analyses that performance on several cognitive domains was associated with longitudinal decline in gait performance. However, the proportion of participants without follow-up gait assessments was high (60.1%). Future studies specifically designed to examine the association between performance on several cognitive domains and longitudinal decline in gait are warranted to rule out that the observations in our exploratory analyses were affected by selective attrition. In addition to etiologic research, studies aiming to develop a population-feasible

screening algorithm for individuals at high risk of dementia may primarily complement this with gait speed, which can easily be assessed on a wide scale and is associated with both cognitive decline and dementia.[3] Gait speed is commonly used to determine current functional health status and predict a broad spectrum of health outcomes, such as functional decline, potential for rehabilitation and mortality.[39] In the coming years, prospective cohort studies with quantitative gait assessments may also accrue sufficient follow-up to examine the association between gait and other common disorders neurodegenerative syndromes in the elderly population, such as parkinsonism (including PD) and normal pressure hydrocephalus.

Five methodological issues of this study warrant consideration. First, we only had two cognitive assessment points, and the second cognitive assessment took place near the end of dementia follow-up. As a consequence, we could not investigate non-linear change over time of gait and cognitive functioning in individuals who were later diagnosed with dementia. Second, 24% of participants did not participate in the follow-up cognitive assessment. Participants in the subgroup with two cognitive assessments were on average slightly younger, more highly-educated, and had slightly better baseline gait and cognitive functioning than the total at-risk population. We cannot rule out that we overestimated some of the hazard ratios due to non-participation at the baseline or follow-up cognitive assessments of individuals with poor gait who were not at increased risk of cognitive decline or dementia (e.g., hip osteoarthritis). Conversely, non-participation of individuals with poor gait and an increased risk of cognitive decline or dementia (e.g. individuals with mild cognitive impairment) would have yielded underestimates of hazard ratios. Third, our study was underpowered to compare effect estimates of gait domains for subtypes of dementia. Specific

quantitative gait domains may be associated with different subtypes of dementia,[40] and may similarly have distinct associations with specific subtypes of dementia. The majority of patients with dementia in the community have mixed pathology, often including Alzheimer's Disease pathology as well as coexisting pathologies such as cerebrovascular lesions.[41-46] Clinically distinguishing dementia subtypes has proven challenging if not impossible in the light of the multitude of pathologies that co-occur in the elderly population. This is particularly troubling in a population-based setting, since 90% of dementia patients in the population are diagnosed after the age of 70 years. As a consequence, the outcome of most population-based longitudinal studies of the prediagnostic phase of dementia (including this study) is the dementia syndrome. We note that our diagnostic approach of both dementia and subtypes of dementia is similar to other large, population-based studies.[47] Fourth, we only assessed gait under single-task conditions, and the battery of cognitive tests we used was not comprehensive. In individuals with mild cognitive impairment, associations of gait with incident dementia are amplified if gait is assessed under dual-task conditions,[48] and a similar pattern may apply to cognitively unimpaired individuals. Fifth, we used multiple imputation to avoid loss of data on baseline gait performance, as 10% of participants did not complete the baseline tandem walk, turning walk or one or two cognitive tasks. We did not systematically record the reason for these missing data. The subgroup of individuals with incomplete data were older, more commonly female, and less commonly highly-educated. We are not sure whether these subgroup differences explain any possible systematic difference between the missing values and the observed values. Therefore, we are unsure whether data were Missing At Random or Missing Not At Random.[49]

In conclusion, our findings suggest that poor performance on several independent gait domains precedes cognitive decline and incident dementia.

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Chapter 3.4

Motoric Cognitive Risk syndrome

ABSTRACT

The Motoric Cognitive Risk syndrome (MCR) has emerged as a potential prodrome for dementia, but its determinants remain largely uninvestigated. We investigated risk factors, brain imaging correlates, objective cognitive functioning, and short-term prognosis of MCR in a population-based cohort. Between 2010 and 2014 we assessed gait speed and subjective cognitive complaints to define MCR in 3,144 mobile, non-demented individuals (mean age 70.6 years, 54.3% women) in the Rotterdam Study. We determined associations with MCR of demographic and anthropometric risk factors of dementia (hypertension, hypercholesterolemia, diabetes mellitus, body-mass index, *APOE* genotype, smoking), and imaging markers on MRI (brain volume, markers of small vessel disease, and white matter integrity). Subsequently, we studied cross-sectionally the association of MCR with performance on objective cognitive testing (Stroop, Letter Digit Substitution, Verbal Fluency, Purdue Pegboard, Word Learning), and longitudinally with risk of dementia and death during an average follow-up of 3 years. At baseline, 314 (10.0%) individuals had MCR, which was associated with all demographic and anthropometric risk factors of dementia. Compared to unaffected individuals, individuals with MCR generally had smaller total brain volume, higher prevalence of lacunar infarcts and white matter lesions, and worse white matter integrity. Individuals with MCR also performed worse on cognitive testing ($p<0.001$ for all tests) and had an increased risk of incident dementia (hazard ratio [HR]=3.27, 95% confidence interval [1.10; 9.70]) and death (HR=1.79 [1.14; 2.82]). In conclusion, MCR shares similar risk factors and findings on brain imaging as clinical dementia, and MCR is associated with the risk of dementia. These findings suggest that MCRmight contribute to the identification of elderly at high risk of dementia in the general population.

BACKGROUND

Pathological changes in the brain may precede clinical symptoms of dementia by decades.[1] At time of diagnosis, pathology is typically so far advanced that putative disease-modifying interventions have no substantial effect.[2] This highlights the need for the identification of individuals before they are demented.

Subjective cognitive complaints are associated with an increased risk of incident dementia[3] and, since they are easily assessable on a community-wide scale, may serve as part of a screening algorithm for dementia in the future. In addition, impaired motor function is increasingly recognized as an important feature of deteriorating brain function in prediagnostic dementia.[4-6] Therefore, an integrated algorithm combining easily assessable objective measures of motor function with subjective cognitive complaint assessment may identify subgroups of prediagnostic dementia patients. This led to the definition of the Motoric Cognitive Risk syndrome (MCR), which is a combination of slow gait and subjective cognitive complaints in mobile elderly.[7] Recently, MCR was shown to be a potential predictor of dementia in several cohorts.[7,8]

However, several important issues with regard to MCR require further clarification. First, it remains unclear whether risk factors for dementia, such as smoking and *APOE* genotype, are also associated with MCR, and to what extent these risk factors are differentially associated with separate MCR components (i.e. cognitive complaints or slow gait speed). Second, although two small studies found associations of MCR with gray matter volumes and lacunar infarcts in the frontal lobe,[9,10] brain imaging determinants of MCR remain largely unexplored. Third, it is unclear how various definitions of MCR affect its association with objective cognitive functioning and short-term risk of dementia and death.

Therefore, we investigated risk factors and brain correlates of MCR and MCR component groups in a large cohort of community-dwelling elderly. Furthermore, within the same population, we studied associations of MCR and MCR component groups with objective cognitive functioning and the short-term risk of dementia and death.

METHODS

Setting

The study was embedded in the Rotterdam Study, a large, prospective, population-based study in the Netherlands.[11,12] In 1990, all inhabitants of the well-defined Ommoord district in the city of Rotterdam who were aged 55 years and older were invited to participate, and 7,983 individuals agreed (first subcohort). In 2000, all inhabitants who had become 55 years of age and older, or moved into the study district since the start of the study were invited to be included in the Rotterdam Study, and 3,011 agreed (second subcohort). The cohort was further extended in 2006 (third subcohort) to a total of 14,926 participants (overall response 72.0 %). By 2014, the first subcohort had a total of five visits, whereas the second subcohort had three visits and the third subcohort had two.

In line with previous studies,[7-10,13-16] we defined MCR as the combination of slow gait and $\geq$ 1 subjective cognitive complaint(s) in older individuals (aged $\geq$60 years) without dementia or mobility disability (identified as walking with a mobility aid [n=14]). Electronic gait assessment was introduced in 2009, and as of March 2014, we had performed gait assessments in 3,285 individuals who were

aged 60 years or older. In 3,147 (96%) of the remaining individuals, we also assessed cognitive complaints. We will refer to this round of assessments as "baseline".

In these participants, we assessed risk factors, brain imaging correlates and objective cognitive functioning. Subsequently, we followed these individuals for the onset of dementia or death until January 1, 2016. Study follow-up for dementia was virtually complete (98%).[17]

Motoric Cognitive Risk (MCR) syndrome

In line with previous studies,[7-10,13-16] we defined MCR as the combination of slow gait ($\leq$ -1 SD age- and sex specific gait speed) and $\geq$ 1 subjective cognitive complaint(s) in older individuals (aged $\geq$60 years) without dementia or mobility disability.

Gait speed: We previously provided a detailed description of gait assessment methods (**Chapter 2.1**). In short, we used a 5.79 m long electronic walkway with pressure sensors (GAITRite Platinum; CIR systems, Sparta, NJ: 4.88 m active area; 120 Hz sampling rate) to assess gait. Participants performed a standardized protocol comprising three walking conditions: 8 normal walks, 1 turning walk and 1 tandem walk. For this report, we only used data from the normal walks, in which participants were asked to walk at their usual pace across the walkway, including 1 practice walk that we did not use for gait speed calculations. We calculated the average speed of the remaining 7 walks and will refer to this as 'gait speed'. Analogous to previously published MCR studies,[7,8,16,18] slow gait was defined as walking speed of -1 standard deviation (SD) or lower compared to age- and sex

specific means. We used a linear regression model based on age and sex to predict gait speed, and subsequently calculated the standardized residual gait speed for each study participant.

Subjective cognitive complaints: Subjective cognitive complaints were evaluated byinterview. This interview included three questions on memory ("Do you have more difficulty to remember things?"; "Are you frequently on your way to do something, and then forget what you had intended to do?"; "Do you have difficulty finding the right words while talking?"), and two questions on everyday functioning ("Do you have difficulty managing finances?"; "Do you have problems using a telephone?"). We scored subjective cognitive complaints positive when a subject answered "yes" to at least one of these five questions. Our definition of the subjective complaint criterion for MCR is analogous to our previously published definition of mild cognitive impairment (MCI),[19] with one exception: we did not use a question on difficulty getting dressed, since this may indicate mobility problems and was therefore considered an exclusion criterion for MCR in previous studies.[7,8,16,18]

Assessment of determinants

Demographic and anthropometric risk factors

Determinants of Motoric Cognitive Risk syndrome were assessed during the baseline home interview and center visits using standardized questionnaires, physical examinations and venous blood drawing. Smoking was assessed during home interviews and study participants were subsequently categorized as current, former and never smokers. Participants were also questioned for current

medication use. This included use of non-steroidal anti-inflammatory drugs (NSAIDs), lipid-lowering drugs (ATC-code C10), as well as calcium-channel blockers, beta-blockers, and other antihypertensive drugs (C02, C03, C07, C08, and C09). Educational level was also assessed and categorized as primary education, lower/intermediate general education or lower vocational education, intermediate vocational education or higher general education, and higher vocational education or university.[20]

Blood pressure was measured by using a random-zero sphygmomanometer at the right brachial artery in sitting position after a 5-minute rest, and hypertension was diagnosed if the mean of two measurements exceeded 140/90mmHg or if an individual used antihypertensive medication. Diabetes mellitus was defined as the use of blood glucose-lowering medication or a fasting serum glucose level ≥7.0 mmol/L.Hypercholesterolemia was defined as the use of lipid-lowering medication or serum total cholesterol level ≥6.2 mmol/L. APOE genotype was determined using polymerase chain reaction on coded DNA samples, and APOE-ε4 status was dichotomized into ≥1 vs. 0 ε4 allele(s).

Neuro-imaging markers

We performed a multi-sequence MRI protocol on a 1.5-Tesla scanner (GE Healthcare) in 3,067 of 3,144 participants.[21] We excluded 124 scans because of technical reasons (such as scanning artifacts or excessive motion) and 92 scans because of the presence of cortical infarcts (as tissue loss and gliosis surrounding cortical infarcts may cause unreliable white matter lesion segmentations). The mean time interval between gait assessment and magnetic resonance imaging (MRI) brain imaging was 0.3 year.

The full MRI protocol has been described in detail before.[21] Briefly, the protocol included T1-weighted (T1w) sequence, a proton density-weighted (PDw) sequence, and a fluid-attenuated inversion recovery (FLAIR)sequence.[21] Diffusion tensor imaging (DTI) scans were also obtained, using a 2D acquisition and EPIreadout.[22] For the quantification of cerebrospinal fluid, gray matter volume, white matter volume, and white matter lesion (WML) volume, we used an automated tissue segmentation method, based ona k-nearest-neighbor brain tissue classifier algorhitm,[23] which was extended with WML segmentation.[24] Total brain volumewas defined as the sum of total gray matter volume,white matter volume, and WML volume. Using the DTI scans, we computed global fractional anisotropy (FA) and mean diffusivity (MD) in the entire normal-appearing white matter as measures of microstructural integrity.[21,22] Lacunes were rated on FLAIR, proton-density-weighted andT1-weighted sequences, and were defined as focal lesions ≥3mm and <15mm in size, with the same signalintensity as cerebrospinal fluid on all sequences anda hyperintense rim on the FLAIR (when located supratentorially).[21] Infarcts showing involvement of graymatter were classified as cortical infarcts. We could not study associations between MCR and cerebral microbleeds because the vast majority of scans used in this report had not been rated for microbleeds yet.

Assessment of Outcomes

Objective cognitive function

We used the abbreviated Stroop color word test (Stroop),[25] Letter Digit Substitution Test (LDST),[26] Word Fluency Test (WFT),[27] Purdue Pegboard test (PPT),[28] and 15-Word List Learning Test (WLT) to assess objective cognitive

functioning. We previously published a detailed description of our assessment methods of objective cognitive functioning.[29]

Ascertainment of dementia

A detailed description of assessment methods has previously been published.[30] In short, participants were screened for dementia at baseline and during follow-up rounds using a three-step protocol.[31] All participants underwent the Mini Mental State Examination (MMSE)[32] and the Geriatric Mental Schedule (GMS) organic level[33]. Individuals with MMSE<26 or GMS>0 were subjected to the Cambridge Examination for Mental Disorders of the Elderly.[34] Additional information was obtained from routinely performed in-person neuropsychological examination, and the total cohort was continuously monitored for dementia through computerized linkage of medical records from general practitioners and the regional institute for outpatient mental healthcare with the study database. Available neuroimaging data were used when required for establishing a diagnosis.

For all suspected cases of dementia, a consensus panel led by a consultant neurologist (PJK), decided on the final diagnosis in accordance with standard criteria for dementia (DSM-III-R), AD (NINCDS-ADRDA), and vascular dementia (NINDS-AIREN). We followed the remaining individuals for the onset of incident dementia and death until January 1, 2015 (first subcohort), 2014 (second), or 2013 (third). For 239 individuals, we had no follow-up for dementia after their center visit; for the remaining 2,905 participants, follow-up was complete.

Statistical analysis

Chapter 3.4

To examine age- and sex-specific gait distribution in our population, we plotted the observed gait speed of each participant in sex strata against their age, and fitted the smoothed age- and sex-specific mean and cut-off (i.e., -1 SD) gait speed.

We used logistic regression models to associations of vascular risk factors and MRI markers (total brain volume, gray matter volume, white matter volume, lacunar infarcts, WML volume, FA, MD) with MCR. Analyses were adjusted for age, sex and study subcohort, and MRI analyses were additionally adjusted for vascular risk factors and intracranial volume. The distribution of white matter lesion volume was left-skewed, so we added 1 to all values and performed a log-transformation. All MRI measures (except lacunar infarcts) were standardized and, if lower scores indicated worse brain (micro-)structure, inverted to facilitate direct comparison of effect sizes. In additional analyses, we repeated FA and MD analyses after additional adjustment for white matter volume and white matter lesion volume, to explore whether white matter integrity was associated with MCR independently of these measures.

Furthermore, we studied associations of MCR with objective cognitive functioning, with adjustment for age, sex, education, and subcohort. We standardized cognitive functioning tests and used principal component analysis to obtain a Global cognition score for each participant, as previously described in detail.[35] Of the Stroop and WLT tests, we used data from the most complicated task of each (i.e., the interference task for Stroop and the 15-minute delayed recall task for WLT) in the calculation of Global Cognition to prevent highly correlated tasks distorting factor loadings in the principal component analysis. Separately, we used Cox proportional hazards models with adjustment for age,

sex, education, and study subcohort to obtain hazard ratios for incident dementia and mortality while at risk of dementia.

Next, in exploratory analyses, we classified participants into 4 "MCR component groups" to estimate to which extent associations of risk factors and MRI measures with MCR, and of MCR with cognitive functioning and incident dementia, were attributable to separate MCR components: individuals with only cognitive complaints, individuals with only slow gait, individuals with MCR, and unaffected individuals (i.e., normal gait speed and no cognitive complaints).

RESULTS

Risk factors of MCR

The mean gait speed in the population was 118 cm/s. Men generally had a slightly higher gait speed than women across the age-span, and the mean gait speed declined proportionally in men and women with advancing age.(*data not shown*) Cognitive complaints were present in more than half of the population (n=1929; 61%), whereas 460 (14.6%) individuals had slow gait. In total, 314 individuals fulfilled MCR criteria.(*Table 1*) Individuals with MCR were generally lower educated and were more frequently smokers. In addition, they more often had hypercholesterolemia, diabetes, hypertension and variant *APOE*-ε4 alleles, although the latter two differences were not statistically significant. Furthermore, their BMI levels were generally higher.

Compared to unaffected individuals, the group of individuals with only cognitive complaints contained more women, smokers and individuals with variant *APOE*-ε4 alleles, whereas the group of individuals with only slow gait contained more

males and individuals with hypertension, hypercholesterolemia and diabetes, and these individuals had higher BMI levels.(*data not shown*) In addition, both component groups were lower educated than unaffected individuals, and this difference was most distinct for individuals with only slow gait.

MRI determinants of MCR

Of volumetric brain measures, smaller total brain volume was significantly associated with MCR, whereas smaller gray and white matter volumes were non-significantly associated with MCR.(*Table 2*) Both measures of focal pathology (lacunar infarcts and larger white matter lesion volume) were associated with MCR. Similarly, both measures of worse white matter integrity (smaller fractional aniotropy and larger median diffusivity) were associated with MCR. After additional adjustment for white matter volume and white matter lesion volume, FA was no longer associated with MCR (odds ratio OR=1.02, 95% confidence interval [0.93; 1.13]) or MD (OR=0.99 [0.88; 1.11]).

Interestingly, individuals with only subjective cognitive complaints had smaller total brain, white matter and gray matter volumes than unaffected individuals.(*Table 2*) However, there were no significant differences between these groups regarding measures of focal brain pathology and white matter integrity. Individuals with slow gait only had non-significantly smaller volumetric brain measures than unaffected individuals, as well as higher white matter lesion volume, and worse white matter integrity. There were no differences between these groups for lacunar infarcts.

Table 1 | Risk factors of Motoric Cognitive Risk Syndrome.

Characteristic	no MCR (n=2830)	MCR (n=314)	Model I OR (95% CI)	Model II OR (95% CI)
Female, n (%)	1537 (54.3)	170 (54.1)	1.00 (0.98; 1.02)	1.01 (0.77; 1.32)
Age, years (SD)	70.4 (7.3)	72.2 (8.5)	1.003 (1.001;1.006)	1.039 (1.011;1.066)
(Intermediate-)higher education, n (%)	1454 (51.9)	135 (43.8)	0.73 (0.56; 0.93)	0.82 (0.63; 1.07)
Ever smoking, n (%)	1822 (64.5)	223 (71.0)	1.36 (1.05; 1.77)	1.27 (0.96; 1.68)
Hypertension, n (%)	2077 (73.5)	254 (80.9)	1.38 (1.02; 1.89)	1.06 (0.74; 1.55)
Hypercholesterolemia, n (%)	1748 (62.1)	221 (70.8)	1.41 (1.10; 1.84)	1.19 (0.88; 1.62)
Diabetes, n (%)	233 (8.6)	47 (15.4)	1.95 (1.37; 2.72)	1.68 (1.16; 2.39)
≥1 APOE-ε4 risk allele, n (%)	730 (27.4)	94 (31.6)	1.25 (0.96; 1.61)	1.39 (1.05; 1.81)
Body mass index, kg/m^2	27.2 (3.9)	28.7 (4.8)	1.09 (1.06; 1.12)	1.09 (1.05; 1.12)

For continuous characteristic, mean value is presented. MCR, motoric cognitive risk syndrome; SD, standard deviation; n, number of participants; APOE, apolipoprotein E; HDL, high-density lipoprotein.

**Education was categorized as [intermediate or higher vocational education, or university] vs. [primary education only or lower vocational education].*

Model I: differences between individuals with and without the Motoric Cognitive Risk Syndrome were adjusted for age, sex and study subcohort. Age was only adjusted for sex and study subcohort; sex was only adjusted for age and study subcohort. Model II: additional adjustment for all other determinants.

MCR, cognitive functioning and prognosis

Individuals with MCR had worse objective cognitive functioning across cognitive tests and for Global cognition than individuals without MCR (p for all<0.001).

Table 2 | MRI determinants of Motoric Cognitive Risk syndrome and its components.

MRI determinant	MCR analysis (ref: no MCR*)	MCR component analysis (ref: unaffected)		
	MCR	Cognitive complaints only	Slow gait only	MCR
Total brain volume (per SD decrease)	1.22 (1.00; 1.49)	1.14 (1.02; 1.27)	1.23 (0.99; 1.54)	1.20 (1.01; 1.42)
Gray matter volume (per SD decrease)	1.14 (0.96; 1.33)	1.09 (0.97; 1.22)	1.17 (0.92; 1.47)	1.18 (0.99; 1.40)
White matter volume (per SD decrease)	1.10 (0.92; 1.28)	1.14 (1.02; 1.27)	1.23 (0.97; 1.56)	1.15 (0.96; 1.37)
WML volume (per SD increase)	1.34 (1.15; 1.55)	0.93 (0.84; 1.03)	1.27 (1.01; 1.60)	1.25 (1.05; 1.47)
Lacunar infarcts (present vs. absent)	1.79 (1.12; 2.79)	0.78 (0.51; 1.20)	0.95 (0.39; 2.34)	1.53 (0.87; 2.70)
Fractional aniotropy (per SD decrease)	1.15 (1.01; 1.32)	0.94 (0.86; 1.03)	1.44 (1.17; 1.78)	1.16 (0.99; 1.35)
Median Diffusivity (per SD increase)	1.26 (1.08; 1.46)	0.92 (0.82; 1.03)	1.57 (1.24; 1.98)	1.21 (1.02; 1.44)

*Odds ratios (95% confidence intervals) of MRI determinants for Motoric Cognitive Risk syndrome and its components. MCR, motoric cognitive risk syndrome. Ref, reference. Values of all MRI determinants (except lacunar infarcts) were standardized. Analyses were adjusted for age, sex, education, smoking, hypertension, hypercholesterolemia, diabetes, APOE genotype, body mass index, total incracranial volume and study subcohort. *Including individuals with subjective cognitive complaints only or slow gait only.*

During follow-up (mean 3.0 years), 41 individuals were diagnosed with incident dementia and 193 individuals died while at risk of dementia. MCR was associated with the hazard of death (hazard ratio [HR]=1.67, 95% confidence interval [1.15;

2.42]) and, albeit non-significantly, with the risk of dementia (HR=1.79 [0.86; 3.71]).Compared to unaffected individuals, MCR was associated with an increased short-term risk of incident dementia and death.(*Table 3*)

Table 3 | Hazard of dementia or death.

Group	N at risk	N incident dementia	HR for dementia (95% CI)	N death	HR for death (95% CI)
MCR vs. no MCR*	*2905*	*41*	*1.86 (0.90; 3.86)*	*193*	*1.67 (1.15; 2.42)*
Unaffected	982	5	(ref)	41	(reference)
Subjective cognitive complaints only	1492	23	2.33 (0.87; 6.22)	87	0.96 (0.65; 1.41)
Slow gait only	130	2	2.07 (0.39; 10.91)	20	2.48 (1.42; 4.32)
MCR	291	11	3.27 (1.10; 9.70)	45	1.79 (1.14; 2.82)

Analyses were adjusted for age, sex, education, study subcohort and competing risk of death. N, number. HR, hazard ratio. CI, confidence interval.
**Including individuals with subjective cognitive complaints only or slow gait only.*

MCR components

Compared to unaffected individuals, individuals with only cognitive complaints had worse scores on WFT (p<0.001), Stroop (p=0.009), WLT (p=0.017) and Global cognition (p=0.002) but not on LDST (p=0.135) and Purdue (0.602), whereas individuals with slow gait had worse scores on Global cognition and each cognitive functioning test (p<0.001 for all) except WLT (p=0.681).(*Table 2*) Compared to individuals with MCR, differences with individuals with only slow gait were only statistically significant for WLT (p=0.021), whereas differences with

individuals with only cognitive complaints were all statistically significant ($p<0.005$ for all).

Alternative definitions of MCR

Using a broader definition for the slow gait speed complaint component of MCR, the percentage of individuals with MCR became much larger (31.5%) than with the definitions used in the main analyses. At the same time, the association between MCR and global cognition only attenuated slightly,(*data not shown*) and the hazard ratio for incident dementia of individuals with MCR compared to unaffected individuals became even higher (HR=5.93 [1.38; 25.51]) than in the main analysis.

Separately, using a stricter definition for the cognitive complaint component of MCR led to a much smaller percentage of individuals with MCR (1.3%) than with the original definition. Simultaneously, associations of MCR with Global cognition (*data not shown*) and incident dementia (HR= 3.77 [1.00; 14.25]) became more distinct. Under this definition, the proportion of individuals with only cognitive complaints was also much smaller, yielding stronger associations of this group with Global cognition and incident dementia.(*data not shown*)

DISCUSSION

MCR has similar risk factors and brain imaging determinants as dementia and is associated with objective cognitive functioning as well as with incident dementia. Taken together, our findings confirm MCR as a predementia syndrome and provide new insight into its brain pathologic correlates.

Before interpreting the results of our study, a few limitations should be noted. First, although we studied a large population-based sample, we observed a relatively low number of incident dementia cases because the mean follow-up for dementia was short (mean 3 years). As a consequence, we lacked statistical power to study subtypes of dementia and directly compare effect estimates for AD vs. vascular dementia, and to investigate potential effect modifiers that could allow for further stratification of dementia risk. Second, the vast majority of Rotterdam Study participants are Dutch Caucasians, potentially limiting the generalizability of our findings to other ethnic and cultural groups, especially those with lower gait speed. Strengths of our study include its population-based design and the detailed assessment of risk factors, brain imaging correlates, and objective cognitive functioning. In addition, we used several questions to assess subjective cognitive functioning, covering both memory and other cognitive domains. Importantly, all analyses were conducted within a single, well-defined population-based cohort, using consistent methods.

Similar to previous studies,[7,8,14,16] elderly with MCR in our sample more frequently had hypertension, diabetes and low education levels than their peers. In addition, we observed that individuals with MCR more frequently smoked, which is in line with a previous non-significant observation in Japanese elderly with MCR,[14] and more commonly had hypercholesterolemia, for which no risk estimates had been reported in previous studies. Furthermore, we identified differential risk profiles for MCR component groups: individuals with slow gait speed only more frequently had hypertension, hypercholesterolemia, diabetes mellitus as well as higher BMI levels, whereas the group with cognitive complaints contained more women, variant *APOE* carriers and smokers.

Furthermore, we observed associations of volumetric brain volumes, focal brain lesions, and white matter integrity with MCR. Interestingly, effect sizes of the associations of gray and white matter volumes with MCR were similar. This differs from a previous, memory clinic-based study of similarly aged elderly,[9] which found an association of gray matter volume, but not white matter volume, with MCR. The latter may be explained by differences in study populations: the remarkably low prevalence of diabetes mellitus among participants of that study (2%) suggests that the study population on average had less vascular risk than a general population. As a consequence, the proportion of individuals with cognitive decline due to predominantly vascular pathology was presumably lower than in population-based studies such as the Rotterdam Study. Furthermore, in our study, effect sizes of the associations of focal lesions (white matter lesion volume and lacunar infarcts) with MCR were remarkably high. This extends finding from a smaller previous study (n=139, including 38 with MCR), which found a borderline association of MCR with lacunar infarcts.[10] Future studies can build on our findings by focusing on the prevalence of regional and tract-specific differences in brain (micro-)structure and focal lesions, and on the predictive value of MCR for longitudinal worsening of subclinical brain pathology.

MCR was strongly associated with a wide spectrum of objective cognitive functioning in our sample, which is in line with a previous study.[8] Interestingly, we observed a similar pattern of diffusely worse objective cognitive functioning in individuals with slow gait only except on a memory test, for which this group performed similarly as unaffected individuals. Conversely, although differences between unaffected individuals and individuals with cognitive complaints only were generally smaller, the latter group did have worse scores on a memory test. This suggests that the group of individuals with MCR contains individuals with

subtle cognitive deficits in diverse domains. In line with previous studies[7-10,13-16] we used a relatively narrow definition of slow gait, and our definition for cognitive complaints was broader, which led to a large difference in the prevalence of these components (15% vs. 61%) and prohibited a direct comparison of their effect sizes. Currently, there are no published, absolute lifetime risk estimates for dementia in MCR patients. However, assuming a remaining lifetime dementia risk of 20% in community-dwelling individuals aged 65 years or older,[36] and a long-term hazard ratio of 2 for individuals with MCR,[8] approximately 40% of all MCR patients would eventually develop clinical dementia. This high proportion would justify further clinical work-up in individuals with MCR, so that high-risk individuals could be monitored for symptom onset to receive early symptomatic treatment or, alternatively, be enrolled in preventive or therapeutic trials. In the future, once treatment options become available that can effectively slow down or halt disease progression, early identification of neurodegenerative disease patients would enable timely initiation of disease-modifying therapies. However, at the same time, it is important to realize that MCR is not equivalent to a predementia diagnosis, since more than half of individuals with MCR will probably never convert to dementia. Furthermore, absolute dementia risk estimates for the middle-long term are much lower (e.g., 10-year risk of dementia is <2% in community-dwelling individuals aged 65 years[36]), meaning that a much larger proportion of individuals with MCR will not convert to dementia in the middle-long term.

In addition, even if all individuals with MCR converted to dementia, they would still represent only a subgroup of prediagnostic dementia cases, since MCR prevalence by its current definition (part of which is $\leq$ -1 SD gait speed) cannot exceed 16% of the elderly population. Because of this, and because of the

generally weak associations of the cognitive complaint component group with brain imaging determinants and objective cognition, we re-examined these associations using two alternative definitions of MCR. When using a stricter cognitive complaint definition, we generally observed stronger associations of MCR with brain imaging determinants and with global cognition. However, the proportion of individuals with MCR was also drastically lower (i.e., 1.5% vs. 10%). Conversely, broadening the slow gait definition only modestly attenuated associations with global cognition and did not alter associations with MRI determinants, while the proportion of individuals with MCR increased vastly (31% vs 10%). Interestingly, the latter alternative definition yielded even stronger effect estimates of MCR for dementia than the original MCR definition and ensured that 59% of all incident dementia patients in our population had first had MCR before dementia diagnosis. This suggests that with a broader MCR definition, MCR assessment would be even more useful to detect incident patients before dementia onset, although this interpretation should be considered preliminary given the small number of incident dementia cases.

In conclusion, MCR has a similar risk profile as dementia and similar brain imaging determinants, and is associated with objective cognitive functioning and incident dementia. Our observations highlight MCR as an easily assessable predementia syndrome that has large overlap with early dementia pathology and subclinical phenomenology. MCR assessment may identify patients from the community before they are demented.

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Chapter 4

Parkinson's Disease: temporal trends

To any student of medical history it is obvious that these experiences are not unique in our own time, but have been repeatedly noted in the past.

Editorial board. The rise and fall of epidemic diseases. Boston Med Surg J 1927; 196:122.

Chapter 4.1

Incidence

4

ABSTRACT

We investigated trends in the incidence of parkinsonism and Parkinson's Disease (PD) over the past 20 years by comparing data from the first (baseline 1990) and second (baseline 2000) subcohort of the population-based Rotterdam Study. From 1990 to 2000, we observed changes associated with a lower risk of PD for some but not all baseline risk factors. Participants in both subcohorts were then followed-up for a maximum of ten years for the onset of parkinsonism, dementia, or death until January 1, 2011. We used Poisson regression models to compare the incidence of parkinsonism, both overall and by cause (PD and secondary causes), and competitive events (incident dementia and death) as well as the mortality of parkinsonism patients in both subcohorts. In the 1990 subcohort, there were 182 parkinsonism cases (84 of whom had PD) after 57,052 person-years. In the 2000 subcohort, we observed 28 parkinsonism cases (10 with PD) after 22,308 person-years. The overall age-and sex-adjusted incidence of parkinsonism was lower in the 2000 subcohort (incidence rate ratio =0.55 [95% confidence interval=0.36;0.81]), and PD incidence declined sharply (incidence rate ratio=0.39 [95% confidence interval 0.19;0.72]). Competitive event incidence rates and mortality rates of parkinsonism patients remained stable. This study suggests that the incidence of parkinsonism in general, and of PD in particular, has decreased between 1990 and 2011.

BACKGROUND

Relatively uncommon before the age of 50, the prevalence of parkinsonism in general and of Parkinson's Disease (PD) in particular increases sharply with age in community-dwelling elderly.[1] As the size of elderly populations increases, PD prevalence would be expected to grow,[2] causing a dramatic projected increase in the burden of PD.[3] However, PD prevalence rates have remained relatively stable over the past decades,[4] suggesting that PD incidence rates may have declined, although this has never been shown empirically.

In the past decades, preventive treatment strategies reducing vascular risk factors at the population level were followed by a decrease in cerebrovascular disease incidence rates.[5] Recently, dementia incidence rates, which may be affected by similar risk factors, were also shown to have declined in community-dwelling individuals.[6] As for PD, a variety of factors are associated with the risk of the disease,[7] but there is little evidence on the causality of these associations. Factors affecting oxidative damage such as serum cholesterol, lipid-lowering medication, and caffeine intake may influence the risk for parkinsonism secondary to cerebrovascular disease as well as the most common causes of dementia, and possibly convey similar protective influences on the risk for PD.[8-10] By contrast, other risk factors such as serum urate and smoking have opposite associations with the risk for PD compared to dementia and stroke.[11-14] An investigation of trends in prevalence of risk factors and subsequent incidence of parkinsonism and PD could provide essential insight into the future burden of PD.

We investigated whether risk factors for and incidence rates of parkinsonism and PD changed over the last two decades by comparing a subcohort of individuals

aged 55 and older in 1990 with a subcohort that started in 2000 from the Rotterdam Study.

METHODS

Study population

This study was performed on participants of the first (baseline 1990) and second (baseline 2000) subcohort of the Rotterdam Study, a large prospective population-based cohort study conducted among middle-aged and elderly in Ommoord, a district of Rotterdam, the Netherlands.[15,16]

Of the 10,275 eligible individuals for the first subcohort (all aged 55 years or older), 7,983 (78%) agreed to participate and signed informed consent statements. Participants were interviewed at home and subsequently invited for in-person screening for parkinsonism at our research center, although 1,030 individuals (13%) did not undergo in-person screening because of refusal, disease or death.[17] For the 2000 subcohort, 4,472 individuals who had become 55 years of age or moved into the study district since the start of the study were invited, of whom 3,011 (67%) agreed to participate and signed informed consent statements. Of these individuals, 550 individuals (18%) did not undergo in-person screening for parkinsonism.

For this report, we excluded individuals with prevalent parkinsonism (including prevalent PD). For PD specifically, we considered individuals with dementia before onset of parkinsonism no longer at risk. Consequently, for PD analyses we excluded 277 individuals with baseline dementia (259 [3.8%] in the 1990 subcohort, 18 [0.7%] in the 2000 subcohort) as well as one individual with

unknown baseline dementia status. There were 128 prevalent parkinsonism cases in the 1990 subcohort (1.8% of those at risk) and 13 in the 2000 subcohort (0.5%). Of prevalent parkinsonism cases, 95 had PD in the 1990 cohort (1.4% of those at risk) and there were 7 prevalent PD cases in the 2000 subcohort (0.3%). Furthermore, 81 individuals retracted informed consent for further data collection during follow-up, leaving 6,752 individuals at risk of parkinsonism (of whom 6,492 were also at risk of PD) in the 1990 subcohort and 2,440 (2,422 for PD) in the 2000 subcohort. Follow-up evaluation from study entry was virtually complete as of January 1, 2011.

Baseline screening and characteristics

At baseline, we used a two-phase design to identify individuals with parkinsonism or PD.[17,18] First, all participants were first screened at the research center for signs of parkinsonism, including standardized, bilateral assessments of hypo-/bradykinesia, resting tremor, postural instability, and cogwheel rigidity. Second, individuals who screened positive received a structured clinical workup (Unified PD Rating Scale [UPDRS]) by a research physician to establish parkinsonism. We obtained additional information from medical records of specialists and general practitioners. Individuals who were suspected of having PD were further evaluated by an experienced neurologist (either in-person, using a recorded video of their visit to the research center, or using a case-report).

We also studied the baseline prevalence of risk factors for PD (smoking, coffee, serum urate, serum cholesterol, and anti-lipid medication use) and of common causes of secondary parkinsonism (primary dementia diagnosis, cerebrovascular

disease, and antipsychotic medication use; see *Online Supplementary Table 1* for details).

Incident parkinsonism and Parkinson's Disease

We used four overlapping modalities to screen for potential parkinsonism during follow-up: in-person screening and interviews during center visits (on average every 4 years), use of antiparkinson medication, and alerts from continuous monitoring of clinical records.[18,19] The proportion of complete data for each modality was very high: on average 93% for data from center visits and 99% for data from medical and pharmacy records.[20] Individuals who screened positive in any of these methods were invited for a UPDRS examination by a research physician to establish parkinsonism. In addition, of all individuals who screened positive, complete medical records were studied and case reports were drawn up covering all potentially relevant information to establish presence and cause of parkinsonism. These case reports were evaluated by a panel led by an experienced neurologist, and the neurologist made the definitive diagnosis. In the process of establishing a diagnosis of parkinsonism, the neurologst did not have access to data on risk factors that were routinely assessed in all Rotterdam Study participants.

Parkinsonism was defined as at least one of: 1) the presence of hypo- or bradykinesia in combination with at least one other cardinal sign (resting tremor, rigidity or postural imbalance) as observed by any physician; 2) a clinical diagnosis of parkinsonism by a neurologist or geriatrician (in the case that motor examination details were not available). We further considered a clinical diagnosis

of parkinsonism by other physicians as possible parkinsonism, however, these individuals were not considered PD cases.

PD was diagnosed after exclusion of parkinsonism associated with preexistent dementia, use of anti-dopaminergic drugs, cerebrovascular disease, multiple system atrophy, progressive supranuclear palsy, and evidence for other rare causes (e.g., corticobasal degeneration), in individuals with at least one of: 1) a clinical PD diagnosis by a neurologist or geriatrician; 2) positive response to dopaminergic treatment. Individuals with parkinsonism who did not fulfill PD criteria were considered secondary parkinsonism cases. The diagnostic criteria for secondary parkinsonism (e.g. secondary to dementia, medication-induced, vascular, multiple system atrophy, progressive supranuclear palsy) have been reported previously[6,17,21] and are summarized in *Online Supplementary Table 3*. After initial diagnosis, medical records of all incident parkinsonism cases (both PD and secondary) continued to be scrutinized until the end of the study period for new information that could lead to a revision of the diagnosis.

End of follow-up

Person-time at risk of incident parkinsonism ended at the first of the following: a diagnosis of parkinsonism, death, ten years after baseline, or January 1, 2011. Person-time at risk of incident PD ended at the first of any of the above or at onset of dementia. Given the substantial overlap between the four detection methods, the consistently high response rates for follow-up in-person examinations, and the fixed maximum time period of ten years, we considered individuals who were not screened in-person during follow-up rounds still at risk of parkinsonism and PD.

Statistical analysis

We compared the prevalence of risk factors for PD and common causes of secondary parkinsonism in the 1990 and 2000 subcohort using linear regression for continuous variables and logistic regression for dichotomous variables, with adjustment for age and sex**.** Fisher exact test was used for dichotomous variables if a percentage was zero.

We calculated person-years at risk per 10-year age-group by adding each participant's contribution of follow-up time within that age group, noting that individuals could contribute person-time to more than one age group. We obtained incidence rates and rate ratios between subcohorts for any parkinsonism, PD, secondary parkinsonism and a composite of competing events (comprising the first of death or incident dementia) per 10-year age group. We used Poisson regression models with person-time at risk as the offset variable and additional adjustment for sex and start age within each age stratum. We repeated overall parkinsonism incidence rate analyses after excluding possible parkinsonism cases (*Online Supplementary Table 3*). We also repeated overall PD incidence rate analyses after narrowing diagnostic criteria for PD (i.e. excluding patients whose PD diagnosis was confirmed by a neurologist but for whom we had no information on response to antiparkinsonian medication consistent with PD). In sensitivity analyses, we compared age-stratified PD incidence rate ratio estimates obtained by Posisson regression with the same estimates obtained by Fine and Gray's model, which takes into account competitive events (first of death or incident dementia).[22] Separately, we calculated overall incidence rates and incidence rate ratios of parkinsonism by other causes than PD, adjusting for age

and sex. Also, we obtained mortality rates and age- and sex-adjusted mortality rate ratios for parkinsonism patients in both subcohorts, stratified in ten year age strata. For this analysis, the time scale ran from onset of parkinsonism until death, ten years after baseline, or January 1, 2011.

RESULTS

Baseline characteristics of participants at risk of PD are presented in *Table 1*. Compared to the 1990 subcohort, the age distribution in the 2000 subcohort was more skewed toward younger participants (mean age 64.5 in the 2000 subcohort vs.69.3 in the 1990 subcohort; $p<0.001$). After stratification by age group, residual age differences between subcohorts persisted in the 55–64 and 75-84 age strata. The 2000 subcohort generally comprised more ever smokers but less current smokers at the time of study entry. Caffeinated coffee intake increased significantly in the 55–64 and 75-84 age strata and non-significantly in the 65-74 age stratum, but decreased in the eldest individuals. Serum urate levels decreased across three of four age strata, but increased in the 65-74 age stratum. The use of lipid-lowering medication was more prevalent in the 2000 subcohort, and serum cholesterol levels were lower. There were less antipsychotic medication users in the 2000 subcohort. Stroke prevalence remained stable across all strata except for a rise in the youngest participants, whereas dementia prevalence was lower in all age strata, although this difference was not significant in the 65-74 year stratum. Exclusion of individuals with prevalent dementia did not meaningfully alter differences of baseline characteristics between subcohorts.

In the 1990 subcohort, after 57,052 person-years, 182 individuals were diagnosed with incident parkinsonism, and in the 2000 subcohort, 28 incident parkinsonism

cases had occurred after 22,308 person-years. Compared with the 1990 subcohort, parkinsonism incidence rates were lower in the 2000 subcohort (incidence rate ratio IRR=0.55, confidence interval [0.36;0.81]). In line with the age difference at baseline between participants in both subcohorts, individuals with incident parkinsonism in the 2000 subcohort were generally somewhat younger at clinical diagnosis than individuals with incident parkinsonism in the 1990 subcohort (mean age 74.9 vs 78.0 years). Stratification by sex showed declining overall parkinsonism incidence estimates in both women and, although non-significant, in men (IRR in women=0.40 [0.20;0.71]; IRR in men=0.74 [0.42;1.22]). The difference in IRR between women and men was not statistically significant (p for interaction term of subcohort and sex = 0.17). Age-specific parkinsonism incidence was much lower in the highest age category and, albeit non-significantly, in the 65-74 and 75-84 age strata *(Table 2*). The proportion of possible parkinsonism cases among all parkinsonism cases was lower in the 2000 subcohort (7%) than in the 1990 subcohort (30%). After exclusion of possible parkinsonism cases, the overall parkinsonism incidence rate ratio between subcohorts was 0.71 [0.46; 1.08].

Table 1 (part I) | Study population characteristics.

Characteristic and subcohort	Age Stratum, years 55–64 No.	%	Mean (SD)	65–74 No.	%	Mean (SD)	75–84 No.	%	Mean (SD)	≥85 No.	%	Mean (SD)	Overall No.	%	Mean (SD)
No. at risk															
1990 subcohort	2,541	37.6		2,426	35.9		1,367	20.2		418	6.2		6,752	100	
2000 subcohort	1,639	67.2		459	18.8		294	12.0		48	2.0		2,440	100	
Age, years															
1990			60.3 (2.8)			69.7 (2.8)			79.3 (2.8)			88.7 (3.1)			69.3 (9.1)
2000			59.8 (2.5)			69.8 (2.9)			78.9 (2.8)			87.8 (2.7)			64.5 (7.9)
P value[a]			<0.001			0.929			0.024a			0.063			<0.001
Ever smoker[b]															
1990	1,811	72.0		1,611	67.7		707	54.0		140	37.0		4,269	64.9	
2000	1,165	71.3		341	74.6		186	63.5		29	60.4		1,721	70.8	
P value	0.410			0.113			0.003			0.022			0.328		
Current smoker[c]															
1990	727	28.9		520	21.9		210	16.0		45	11.9		1,502	22.8	
2000	436	26.7		85	18.6		34	11.6		2	4.2		557	22.9	
P value	0.046			0.064			0.033			0.036			0.001		

Table 1 (part II) | Study population characteristics.

Characteristic and subcohort	Age Stratum, years 55–64 No.	%	Mean (SD)	65–74 No.	%	Mean (SD)	75–84 No.	%	Mean (SD)	≥85 No.	%	Mean (SD)	Overall No.	%	Mean (SD)
Coffee intake, g/day															
1990			481.3 (305.1)			406.8 (246.5)			337.2 (224.7)			358.2 (226.3)			425.6 (274.6)
2000			562.2 (376.2)			432.3 (379.9)			369.9 (216.8)			281.3 (205.5)			274.6 (509.5)
P value			<0.001			0.125			0.037			0.031			<0.001
Urate, µmol/L															
1990			314.7 (77.8)			322.7 (78.3)			334.6 (87.3)			350.5 (97.5)			324.4 (82.1)
2000			309.7 (74.4)			337.2 (87.5)			323.2 (84.1)			337.2 (87.5)			313.5 (76.1)
P value			0.001			0.029			0.035			0.310			<0.001
Cholesterol, mmol/L															
1990			6.7 (1.2)			6.7 (1.2)			6.4 (1.3)			6.1 (1.3)			6.6 (1.2)
2000			5.9 (1.0)			5.6 (1.0)			5.6 (1.1)			5.5 (1.0)			5.8 (1.0)
P value			<0.001			<0.001			<0.001			0.008			<0.001

Table 1 (part III) | Study population characteristics.

Characteristic and subcohort	Age Stratum, years 55–64 No.	%	Mean (SD)	65–74 No.	%	Mean (SD)	75–84 No.	%	Mean (SD)	≥85 No.	%	Mean (SD)	Overall No.	%	Mean (SD)
Lipid-lower. medication															
1990	75	3.0		74	3.1		11	0.8		2	0.5		162	2.4	
2000	196	12.0		75	16.3		41	13.9		2	4.2		314	12.9	
P value	<0.001			<0.001			<0.001			0.059			<0.001		
Antipsychotic medication															
1990	229	9.0		364	15.0		293	21.5		114	27.3		1,000	14.8	
2000	145	8.8		40	8.7		40	13.6		5	10.4		230	9.4	
P value	0.936			0.003			0.006			0.028			0.001		
Stroke															
1990	24	0.9		60	2.5		80	5.9		29	6.9		193	2.9	
2000	36	2.2		15	3.3		23	7.8		2	4.2		76	3.1	
P value	0.001			0.461			0.162			0.414			0.002		
Dementia															
1990	7	0.3		32	1.3		105	7.7		115	27.5		259	3.8	
2000	0	0.0		3	0.7		11	3.7		4	8.3		18	0.7	
P value	0.047			0.228			0.035			0.012			<0.001		

SD, standard deviation. [a]P values for differences between subcohorts were adjusted for age and sex. [b]Reference category was never smoking. [c]Reference category comprised both former and never smoking. Table 2. Incidence Rate Ratios for Parkinsonism by Age Stratum Among Participants in the Rotterdam Study by Subcohort (2000 Subcohort (2000–2010) Versus 1990 Subcohort (1990–2000)).

Table 2 | Parkinsonism incidence rate ratios in the Rotterdam Study: 2000 subcohort (2000-2010) vs 1990 subcohort (1990-2000).

	1990 Subcohort			2000 Subcohort			2000 vs. 1990 Subcohort
Age, years	No.	Person-time at Risk[a]	Incidence Rate[b] (95% CI)	No.	Person-time at Risk	Incidence Rate[b] (95% CI)	Incidence Rate Ratio[b,c] (95% CI)
55-64	8	11,688	0.68 (0.30; 1.35)	5	8,308	0.60 (0.20; 1.40)	0.87 (0.26; 2.60)
65-74	57	24,501	2.33 (1.76; 3.01)	10	9,659	1.04 (0.50; 1.90)	0.53 (0.25; 1.01)
75-84	82	15,912	5.15 (4.10; 6.40)	12	3,320	3.61 (1.87; 6.31)	0.68 (0.35; 1.20)
≥85	35	4,951	7.07 (4.92; 9.83)	1	1,020	0.98 (0.02; 5.46)	0.14 (0.01; 0.67)

No., number of incident parkinsonism cases. 95% CI, 95% confidence interval. [a]Person-time at Risk is expressed in person-years. [b]Incidence rates and incidence rate ratios are displayed per 1,000 person-years. [c]Incidence rate ratios were adjusted for age and sex; reference (=1.00) is incidence rate in the 1990 subcohort.

As shown in *Table 3*, there was a remarkable decrease in the incidence of PD and parkinsonism due to vascular causes, while the incidence of parkinsonism due to other rare causes increased. There were no significant changes in the incidence of parkinsonism secondary to dementia or multiple system atrophy, whereas the incidence of medication-induced parkinsonism and of individuals without a clear cause for parkinsonism decreased. Incident PD rate ratio estimates remained similar when competing risks were taken into account (*Online Supplementary Table 4*).

Participants in the 1990 subcohort amassed 55,920 person-years at risk of PD, during which 84 individuals developed incident PD. In the 2000 subcohort, there were ten PD cases in 22,224 person-years at risk. The overall incidence of PD was remarkably lower in the 2000 subcohort (IRR=0.39 [0.19;0.72]). As shown in *Table*

4, the incidence of PD decreased across all age groups. Stratified analyses showed a more pronounced but not significantly different overall decline in PD incidence in women than in men (IRR in women=0.18 [0.03;0.58]; IRR in men=0.57 [0.25;1.16]; P for interaction term of subcohort and sex = 0.17). 41 out of 84 PD cases (49%) in the 1990 subcohort and six out of ten PD cases (60%) in the 2000 subcohort fulfilled narrower diagnostic criteria for PD; we had no DaTSCAN-data for any of these patients. Narrowing diagnostic criteria for PD did not alter the observed overall decrease in PD incidence (overall age- and sex-adjusted IRR=0.41 [0.16;0.91]).

In the 1990 subcohort, 281 participants were diagnosed with incident dementia (4%) and 1,521 individuals died (23%) while at risk of PD. Of individuals at risk of PD in the 2000 subcohort, 73 individuals were diagnosed with dementia before parkinsonism onset (3%) and 324 individuals died (13%). Overall, the composite competitive risk was lower in the 2000 subcohort (IRR=0.79 [0.70;0.88]). As shown in *Table 5*, the age- and sex-adjusted competitive event incidence rate estimates for each 10-year stratum were 20-24% lower in the 2000 subcohort than in the 1990 subcohort.

100 incident parkinsonism patients in the 1990 subcohort died after 885 person-years with parkinsonism (mortality rate = 113.0 [92.0;137.5]), whereas in the 2000 subcohort 13 patients with parkinsonism died after 114 person-years with parkinsonism (mortality rate = 113.6 [60.5;194.2]), and the age- and sex adjusted mortality ratio (MRR) was 1.37 [0.71;2.45].

Table 3 | Trends in the Incidence Rate of Parkinsonism by Cause Among Participants in the Rotterdam Study by Subcohort

Cause of parkinsonism	1990 Subcohort			2000 Subcohort			2000 Subcohort vs. 1990 Subcohort
	No. of Cases[a]	Person-Years at Risk	Incidence Rate[b] (95% CI)	No. of Cases[a]	Person-Years at Risk	Incidence Rate[b] (95% CI)	Incidence Rate Ratio[b,c] (95% CI)
Parkinson's disease	84	55,920	1.50 (1.20; 1.86)	10	22,224	0.45 (0.22; 0.83)	0.39 (0.19; 0.72)
Dementia[d]	8	57,052	0.14 (0.06; 0.28)	5	22,307	0.22 (0.07; 0.52)	2.68 (0.80; 8.18)
Medication-induced parkinsonism	19	57,052	0.33 (0.20; 0.52)	1	22,307	0.04 (0.00; 0.25)	0.17 (0.01; 0.86)
Multiple system atrophy	3	57,052	0.05 (0.01; 0.15)	1	22,307	0.04 (0.00; 0.25)	1.15 (0.06; 9.29)
Vascular parkinsonism	7	57,052	0.12 (0.05; 0.25)	0	22,307	0.00 (0.00; 0.17)	0.00 (0.00; 0.00)
Other rare causes[e]	4	57,052	0.07 (0.02; 0.18)	5	22,307	0.22 (0.07; 0.52)	3.76 (0.96; 15.74)
Unspecified	57	57,052	1.00 (0.76; 1.29)	6	22,307	0.27 (0.10; 0.59)	0.42 (0.16; 0.90)
Overall	182	57,052	3.19 (2.74; 3.69)	28	22,307	1.26 (0.83; 1.81)	0.55 (0.36; 0.81)

CI, confidence interval. [a]Number of incident cases of parkinsonism. [b]Incidence rates and incidence rate ratios are displayed per 1,000 person-years. [c]Incidence rate ratios were adjusted for age and sex; the referent was the incidence rate in the 1990 subcohort. [d]The dementia diagnosis comprised all clinical causes of dementia before the onset of parkinsonism except for dementia with Lewy bodies. [e]Other rare causes of parkinsonism included dementia with Lewy bodies and progressive supranuclear palsy (for each: 1 case in the 1990 subcohort and 2 in the 2000 subcohort), tumor (2 in the 1990 subcohort), and corticobasal degeneration (1 case in the 2000 subcohort).

Table 4 | Incidence Rate Ratios for Parkinson's Disease Among Participants in the Rotterdam Study by Subcohort.

Age Stratum, years	1990 Subcohort			2000 Subcohort			2000 Subcohort vs. 1990 Subcohort
	No. of Cases[a]	Person-Years at Risk	Incidence Rate[b] (95% CI)	No. of Cases[a]	Person-Years at Risk	Incidence Rate[b] (95% CI)	Incidence Rate Ratio[b,c] (95% CI)
55–64	5	11,660	0.43 (0.14; 1.00)	2	8,308	0.24 (0.03; 0.87)	0.55 (0.08; 2.58)
65–74	28	24,383	1.15 (0.76; 1.66)	5	9,645	0.52 (0.17; 1.21)	0.51 (0.17; 1.22)
75–84	39	15,472	2.52 (1.79; 3.45)	2	3,273	0.61 (0.07; 2.21)	0.23 (0.04; 0.74)
≥85	12	4,406	2.72 (1.41; 4.76)	1	999	1.00 (0.03; 5.58)	0.33 (0.02; 1.70)

CI, confidence interval. [a]Number of incident cases of Parkinson's Dsiease. [b]Incidence rates and incidence rate ratios are displayed per 1,000 person-years. [c]Incidence rate ratios were adjusted for age and sex; the referent was the incidence rate in the 1990 subcohort.

Table 5 | Age- and Sex-Adjusted Rates and Rate Ratios for Competing Events Among Participants in the Rotterdam Study by Subcohort.

Age Stratum, years	1990 Subcohort			2000 Subcohort			2000 vs. 1990 Subcohort
	No. of Persons[a]	Person-Years at Risk	Incidence Rate[b] (95% CI)	No. of Persons[a]	Person-Years at Risk	Incidence Rate[b] (95% CI)	Incidence Rate Ratio[b,c] (95% CI)
55–64	80	11,660	6.90 (5.40; 8.50)	44	8,308	5.30 (3.80; 7.10)	0.76 (0.52; 1.09)
65–74	412	24,383	17.30 (15.90; 18.70)	124	9,645	13.50 (11.30; 15.90)	0.80 (0.65; 0.98)
75–84	717	15,472	49.90 (47.40; 52.40)	126	3,273	41.60 (35.00; 49.00)	0.79 (0.65; 0.96)
≥85	593	4,406	156.30 (148.90; 164.10)	103	999	118.00 (98.00; 140.80)	0.78 (0.63; 0.96)

CI, confidence interval. [a]Number of individuals with a competing event. Competing events for Parkinson's Disease included death and incident dementia, whichever occurred first. When individuals were diagnosed with dementia without preceding parkinsonism, they were considered no longer at risk of Parkinson's Disease. [b]Incidence rates are displayed per 1,000 person-years. [c]Incidence rate ratios were adjusted for age and sex; the referent was the incidence rate in the 1990 subcohort.

DISCUSSION

Comparing two subcohorts of the population-based Rotterdam Study, we found that the incidence of parkinsonism in the general population has declined over the course of the past twenty years. PD remains the most frequently recognized cause of parkinsonism, and its incidence has decreased sharply in this cohort.

Before this study, three articles had been published on trends in PD incidence in Caucasian populations after 1990, reporting stable incidence rates[23,24] and a small decrease in PD incidence,[25] respectively. However, those studies relied largely on general practitioner records and diagnosis codes, which harbors a high susceptibility for misclassification of cases and cannot distinguish between secondary parkinsonism and PD.[26] Strengths of our study include its population-based design, extensive case-detection methods which yielded detailed clinical information on causes of parkinsonism, and standardized assessment for both parkinsonism and PD throughout the study period. Limitations include the low number of PD cases in the 2000 subcohort and the lack of histologic confirmation of specific pathologies. In addition, our case-finding methods were mainly directed at detecting PD, which may have caused an underestimate of parkinsonism due to other causes than PD in both subcohorts. Also, we note that we lacked data on some known risk factors for PD (e.g. exposure to pesticides) and that our study population comprised almost exclusively Caucasians, which may limit the generalizability of our findings to other populations.

We consider five possible explanations for the remarkable decline in parkinsonism and PD incidence. First, a lower prevalence of risk factors in the 2000 subcohort could have caused a drop in incidence rates of parkinsonism and PD in the following decade. We observed a decrease in serum urate levels, which has been

associated with a higher risk of PD.[12] Apart from in the eldest individuals, we observed an increase in caffeinated coffee intake, which has been associated with a lower risk of PD.[10] Similarly, there was a rise in the use of lipid-lowering medication and a concomitant drop in cholesterol levels, which may beneficially affect the risk for PD.[8,9] As for smoking, prospective cohort studies have consistently shown an inverse association with the risk of incident PD, and a stronger association for current smoking than for former smoking.[14] In this study, we observed a higher prevalence of ever smoking in the 2000 subcohort, but the proportion of current smokers was lower, suggesting that changes in baseline risk profiles were not uniform. More importantly, evidence supporting the causality of the association of above risk factors with incident PD is limited, especially for smoking. In addition, even assuming causal associations with PD for risk factors such as serum urate and caffeine, there is little data on the population attributable risk they represent for PD. To further understand the mechanisms responsible for the decline in parkinsonism and PD incidence, we need high-quality data on trajectories toward the onset of clinical PD.

Second, the decline in parkinsonism and PD incidence could merely reflect a change in application of diagnostic criteria. Although case-detection methods for parkinsonism and PD within the Rotterdam Study remained similar throughout the study period, PD diagnosis in research setting in part relies on exclusion of secondary causes of parkinsonism.[27] Therefore, we examined the prevalence of secondary causes of parkinsonism and the incidence of parkinsonism by cause. The decrease in baseline antipsychotic medication use was paralleled by a decline in the incidence of medication-induced parkinsonism, while the lack of a major, unidirectional shift in prevalence rates of stroke within our population and the previously reported decrease in stroke incidence in the Rotterdam Study[5] were

followed by a decrease in vascular parkinsonism. Furthermore, the increase in rare causes of parkinsonism and the decrease in unspecified parkinsonism may reflect advanced availability of diagnostic modalities and increased understanding of secondary parkinsonism by neurologists. Therefore, we repeated our analyses after excluding patients whose PD diagnosis was confirmed by a neurologist but for whom we had no information on response to antiparkinsonian medication consistent with PD. We observed a similar decline in PD incidence, suggesting that these changes did not account for the decline in PD incidence. We also compared overall parkinsonism incidence between subcohorts after exclusion of possible parkinsonism cases, which rendered the difference between subcohorts no longer significant. However, we note that the incidence rate ratio between subcohorts (0.71) was not vastly different than the estimate when all cases were included (0.55).

Third, increasing awareness of early parkinsonian signs among clinicians could have led to a shift in timing of parkinsonism diagnosis, inducing a spurious decline in parkinsonism incidence rates. Participants were all 55 or older at the time of study entry and were at risk of parkinsonism and PD for a fixed maximum time period (i.e., ten years). Therefore, a left-shift in timing of parkinsonism diagnosis would render relatively young patients to be considered incident cases in the 1990 subcohort and prevalent cases in the 2000 subcohort. However, the prevalence of parkinsonism and PD cases was much lower in the 2000 subcohort, and although the difference in parkinsonism and PD incidence between subcohorts was particularly pronounced in the eldest, incident rate ratios in other strata suggested a decline in parkinsonism and PD incidence rates across all age groups. Similarly, our observation that incident parkinsonism and PD cases in the 2000 subcohort were generally younger at the time of diagnosis was accounted

for by the difference in age at baseline. We also investigated mortality rates of parkinsonism patients, since a left-shift in timing of parkinsonism diagnosis would artificially increase survival time of patients within a fixed maximum time period (in the absence of major changes in parkinsonism treatment practices). We observed similar mortality rates for patients in both subcohorts, although we note that the low number of patients who died limited our power to detect modest overall changes and prevented us from performing subgroup analyses (e.g., PD patients only). Taken together, we found little evidence supporting that a left-shift in timing of parkinsonism diagnosis explains the remarkable decline in incidence rates.

Fourth, selection bias may have occurred. Although standardized and uniform ascertainment methods were used throughout the study period and the follow-up was virtually complete in both subcohorts, the baseline response rate was higher in the 1990 subcohort than in the 2000 subcohort (78% vs 67%). In 2000, most inhabitants of the study area who were aged 65 or older were already enrolled in the 1990 subcohort of the Rotterdam Study (and a further minority had already refused to participate). Therefore, the majority of individuals invited in 2000 were aged 55-64, and a substantial proportion of these invitees was still working. We believe that this may have contributed to the difference in response rates between subcohorts. The dissimilar response rates, as well as the age distribution, may have contributed to the major difference in prevalence of parkinsonism (1.8% vs 0.5%) and PD (1.4% vs 0.3%) between subcohorts, since individuals with greater disability (such as parkinsonism patients) may be less likely to participate in an observational study such as the Rotterdam Study. In the absence of a left-shift in timing of diagnosis, one would expect that the difference in response rate would not directly affect age-specific incidence rates of

parkinsonism and PD, unless the non-responder group in both subcohorts comprised a large proportion of individuals at high risk for clinical parkinsonism and PD. The latter appears unlikely given that some common risk factors that are inversely associated with parkinsonism, and in particular with PD, are associated with higher risk of much more common outcomes in elderly (e.g., smoking and serum urate with cardiovascular disease). However, in view of the lack of data on population attributable risk of these factors for PD, even individuals without any of these known risk factors could theoretically be at high risk for the disease, and their prodromal signs (e.g., depressive symptoms) could have decreased their likelihood of participating in this study.

Fifth, in individuals with preexistent dementia it remains difficult to clinically distinguish PD from parkinsonism that occurs secondary to the disease they already have (often Alzheimer's Disease).[28] We considered individuals with a dementia diagnosis no longer at risk of PD. As a consequence, incident dementia, as well as death, can be considered a competitive event for incident PD. Since underlying risk factors for dementia and mortality may also predispose to PD (e.g. genetic risk factors, oxidative agents), we examined age-specific changes in the incidence rate of competitive events. We observed a decline across all age groups, suggesting that the composite competing risk for PD was higher in the 1990 subcohort. Had the competitive risk remained stable, an even larger decrease in PD incidence would have occurred. Besides, overall parkinsonism and PD incidence rates were lower for women in the 2000 subcohort than in the 1990 subcohort, whereas incidence rates in men declined non-significantly. However, we did not observe statistically significant effect modification by sex, and we could not investigate age-specific differences for men and women separately.

Therefore, we consider our observation on sex-differences preliminary, and this needs to be confirmed by future studies.

In conclusion, this study suggests that parkinsonism rates have declined over the past two decades, largely as a consequence of a sharp drop in the incidence of PD. Further insight into the factors that caused this shift may open the door to protective strategies at the population level.

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Chapter 4.2

Mortality after diagnosis

Chapter 4.2

ABSTRACT

Recent observational studies have drawn attention to the rapid rise in the burden caused by Parkinson's Disease (PD) over the past years, emphasizing that PD is a matter of serious concern for our future generations. A recent report by Public Health England corroborates this message, by providing new insight on trends in deaths associated with neurological diseases in England between 2001 to 2014. The report indicates that mortality associated with PD and related disorders increased substantially between 2001 and 2014. This trend is partially explained by increased longevity in the population. However, it is possible that changes in exposure to risk factors, recent improvements in multidisciplinary care (leading to prolonged survival), and improved diagnostic awareness or improved registration also influenced the observed trend. Furthermore, patients with PD and related disorders were found to die at an advanced age, and the majority die in a care home or hospital, despite a preponderant preference for many patients and their families to spend their last days at home. To combat these concerning observations, future efforts should be focused on providing resources for vulnerable elderly Parkinson patients, avoiding unplanned hospital admissions and out-of-home deaths as much as possible. Possible solutions include a community-based network of specifically trained allied health therapists, personal case managers for Parkinson patients, dedicated Parkinson nursing homes, and improved centralized support services from university clinics to regional community hospitals aimed at facilitating optimal wide-scale care delivery.

BACKGROUND

The burden caused by Parkinson's Disease (PD) has recently increased at the population level.[1,2] Scrutiny of temporal trends in causes of death can offer useful complementary information in this regard. Here, we draw attention to a recently published report by Public Health England on trends in deaths associated with neurological diseases in England between 2001 to 2014,[3] which contains several interesting observations concerning Parkinson's Disease and related disorders (PDRD).(Box 1) We will summarise these findings, and address some implications for future healthcare needs. Specifically, we focus on the frequency, age and place of deaths associated with PDRD, as well as on temporal trends in the frequency of these deaths.

Box 1 | Key observations in the Public Health England report

1. *Mortality associated with Parkinson's Disease and related disorders (PDRD) increased substantially between 2001 and 2014; this trend appears to be partially explained by increased longevity.*
2. *PDRD patients generally die at an advanced age.*
3. *The majority of PDRD patients die in a care home or hospital.*

Causes of death in the ageing population: the Public Health England Report

In the United Kingdom, it has long been a legal requirement to report deaths to the Office of National Statistics, yielding near-complete mortality data on a population-wide scale (https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths). For each deceased individual, healthcare professionals register a single underlying cause of death, as well as up to 15 contributory conditions on

the death certificate. Conditions are coded in line with the International Statistical Classification of Diseases and Related Health Problems.

According to the Public Health England report,[3] 6.590.453 individuals aged 20 years and over died in England between 2001 and 2014. Of these deaths, 366.728 (6%) were associated with at least one neurological condition (listed as the underlying cause or a contributory cause of death). The report also contains data on deaths associated with seven specific groups of neurological conditions: epilepsy; motor neurone disease and spinal muscular atrophy; multiple sclerosis and inflammatory disorders; neuromuscular diseases; traumatic brain and spine injury; tumours of the nervous system; and PDRD. The latter group comprised all disorders characterized by parkinsonism (including atypical parkinsonisms), other extrapyramidal disorders (including chorea), or tic disorder. Of note, dementia (including dementia with Lewy bodies) and stroke were not among the groups of neurological conditions analysed in the above-referenced report, however, they could be recorded as the underlying cause of death on a death certificate.

Common causes of death

The group that was most commonly listed on death certificates between 2001 and 2014 was PDRD; these were mentioned on 31% of deaths associated with a neurological condition as the underlying cause, the contributory cause, or both. PD was the most commonly recorded underlying neurological cause of death (14% of deaths associated with a neurological condition). Remarkably, falls (6%) and pneumonia (3%) were among the 10 most common underlying causes of deaths associated with a neurological condition, while Alzheimer's Disease, vascular dementia and unspecified dementia were each recorded on only 1% of

death certificates. Falls and pneumonia may have occurred as a complication of parkinsonism or dementia in some patients, which implies that the report may have underestimated how often these diseases are the underlying cause of death. Indeed, falls and fall-related injuries are very common in PDRD patients, and hip fractures in this population are associated with high mortality rates.[4,5] Aspiration pneumonia secondary to dysphagia is also a common cause of death for PDRD patients.[6] On the other hand, some misclassification of a diagnosis of PD may have occurred, as a previous study showed that almost one in six patients with a diagnosis of PD in the population did not fulfil strict clinical criteria for the disease.[7] Taken together, these data demonstrate that deaths associated with PDRD are common in the population.

Age and place of deaths

Deaths associated with PDRD were the only group of neurological conditions for which the mean age at death was higher than the overall mean age at death in the population (82 vs. 78 years, data reported for the 2012-2014 period). PD is not exclusively diagnosed in the very elderly,[8] and the high age at death suggests that PD patients live for long periods of time with this condition. Considering the projected rise in life expectancy globally, more resources resources will be needed to take care of elderly patients with PD.

Care homes were the most common place of death among patients with PDRD, occurring in 43% of cases. Notably, healthcare professionals working in these institutions frequently lack PDRD-specific expertise.[9] Perhaps dedicated PDRD nursing homes or PDRD-specific training programs could help to reduce some preventable deaths. Aside from care homes, 41% of deaths associated with PDRD

were recorded in hospitals, 14% occurred at home, and 2% in other places. By comparison, 23% of all-cause deaths and 18% of deaths associated with neurological conditions occurred at home. These data suggest that, despite recent improvements,(14-16) the end-of-life for PD patients is often unplanned and occurring in hospitals or care homes. Although there are undeniably instances when dying in a hospital is more appropriate than dying at home, most patients indicate a preference for dying at home.[10] Family caregivers also typically indicate their own home as the preferred place of care for their relatives towards the end-of-life.[11] Furthermore, the vast majority of hospital admissions of PD patients are unplanned, resulting from either complications of the disease or its treatments or comorbidities.[12,13]

PDRD-specific training of professionals working in the community might prevent some of these unscheduled admissions. For instance, care delivery by physiotherapists with specific expertise in PDRD management was associated with fewer hospital admissions due to fractures, other orthopaedic injuries or pneumonia, as compared to regular care by a generically trained therapist.[14] Further gains may be made by introducing intensive case management for community-dwelling PDRD patients, entailing a personalized, collaborative plan of care, not only for but also with patients and their families. Also, closer collaboration between university clinics and regional community hospitals may improve delivery of optimal patient care, with an emphasis on care delivered close to the patient's home whenever possible, but with seamless access to more remote specialized care whenever needed. Specific areas of collaboration may include peer-to-peer support (e.g. university centers supporting community-based colleagues with less expertise), diagnostics in university clinics for unclear

cases, shared education programs for patients and professionals, and improved regional guideline development.

Temporal trends

The number of all-cause deaths per year gradually declined between 2001 and 2006 and remained relatively stable between 2007 and 2014. Compared to 492,205 deaths in 2001, there were

464.556 in 2014, representing a 6% decline. By contrast, the yearly number of deaths associated with a neurological condition increased steadily from 23,051 in 2001 to 31,925 in 2014, representing a 39% increase over this relatively short time period. The number of deaths associated with PDRD increased from 6,963 to 10,067 during the study period, indicating a marked 45% rise. There were even steeper relative rises in mortality associated with neuromuscular diseases (+83%), epilepsy (+70%), and traumatic brain and spine injury (+64%). Ageing of the population did not fully account for the observed rise in mortality associated with a neurological condition, since age-standardized mortality for deaths associated with a neurological condition only increased by 12%. Age-standardized mortality of deaths associated with PDRD rose by 10%.

The increase in mortality associated with PDRD may be reflective of a rise in the incidence, a longer survival after diagnosis, an increased awareness of coding these conditions on death certificates in England, or a combination thereof. Temporal trends in the incidence of parkinsonism, including its most common cause PD, have varied substantially across populations around the world.[15-18] It is possible that differential temporal changes in exposure to risk factors (e.g

exposure to airborne pollutants, toxins such as pesticides, heavy metals or solvents) have resulted in discrepant trends in the incidence of parkinsonism across populations. Furthermore, we consider the possibility that survival after diagnosis among parkinsonism patients may have increased as a result of improvements in multidisciplinary care during the last decade.[14,19,20] Future studies are warranted to elucidate to what extent such changes may have affected the observations in the Public Health England report.

We also note that age-standardized mortality of almost every group of neurological conditions increased throughout the study period. This suggests that an improved awareness of neurological conditions or a more complete registration of contributory causes on death certificates over time may have contributed to the observed rise in mortality associated with PDRD. Unfortunately, it is not clear whether the number of contributory conditions listed on death certificates changed during the study period.

Implications for future healthcare needs

Health care planning should properly anticipate a possible further rise in mortality associated with PDRD. Efforts should particularly be focused on providing resources for vulnerable elderly patients with these disorders, avoiding unplanned hospital admissions and out-of-home deaths as much as possible. Possible solutions include a community-based network of specifically trained allied health therapists, personal case managers for PDRD patients, dedicated PDRD nursing homes, and improved centralized support services from university clinics to regional community hospitals aimed at facilitating optimal wide-scale care delivery.

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Chapter 5

Parkinson’s Disease: genetic predisposition

This finding of a specific molecular alteration associated with Parkinson’s Disease will facilitate the detailed understanding of the pathophysiology of the disorder.

Mihael H. Polymeropoulos et al. Mutation in the α-Synuclein Gene Identified in Families with Parkinson's Disease. Science. 1997.

Chapter 5.1

Utility of GWAS top hits

ABSTRACT

We investigated whether a risk score based on genetic risk variants implicated in Parkinson's disease (PD) is associated with the risk and improves prediction of incident PD, and whether the risk score is associated with basic activities of daily living (BADL) in healthy individuals. Within the population-based Rotterdam Study, we genotyped 26 independent risk variants implicated in PD and constructed a genetic risk score in 7,167 participants who were free of parkinsonism and dementia at baseline (1990 or 2000). Participants were followed for a maximum of twenty years for the onset of parkinsonism, dementia or death until January 1, 2011 (median follow-up 12.1 years). We studied the relationship between the genetic risk score and incident PD with adjustment for age, sex, smoking and parental history. In an independent sample of 2,997 individuals free of parkinsonism and dementia, we studied whether the PD risk score was associated with impaired BADL. During follow-up (median 12.1 years), 99 individuals were diagnosed with incident PD. The genetic risk score was associated with incident PD (hazard ratio per standard deviation risk 1.25 [95% confidence interval=1.02;1.55]), but did not substantially improve prediction (change in C-statistic 0.687 [0.628; 0.745] to 0.698 [0.635; 0.760], ΔC=0.011 [-0.011;0.033]). The genetic risk score was associated with a higher probability of any impairment in BADL (odds ratio=1.11 [1.00;1.23]). In conclusion, genetic variants implicated in PD are associated with the risk of incident PD in the general population and with impairment in daily functioning in individuals without clinical parkinsonism, but do not improve the clinical prediction of PD.

BACKGROUND

Parkinson's Disease (PD) is the second most common neurodegenerative disorder among the elderly.[1] Clinically, the disease is characterized by parkinsonism, an absence of markers suggestive of other causes, and supportive prospective criteria.[2] Clinical PD is preceded by a prodromal phase during which neurodegeneration has already started, but the signs defining parkinsonism are not present.[3] During this period, individuals often experience a combination of early motor and non-motor signs and symptoms that could affect their daily activities, ranging from subtle movement deficits under challenging conditions to autonomic dysfunction, rapid eye movement sleep behavior disorder, and depression.[4]

Several factors are associated with an altered risk of incident PD, such as environmental risk factors (e.g., smoking, exposure to pesticides) and early clinical features (e.g., anosmia, rapid eye movement behavior disorder).[1,5] However, there is a lack of empirical data on whether these factors can identify a large group of individuals at high risk for the disease from the general population. During the last decade, several studies have suggested a substantial genetic contribution to PD, including the identification of risk-increasing mutations in *GBA* and *LRRK2* that are common in PD patient populations,[6,7] with a large proportion of contributing genes still to be identified.[8] In addition, genome-wide association studies have yielded a total of 28 independent risk variants that are common at a population level, 22 of which are genome-wide significant.[9] Recent case-control studies have shown a risk score based on these variants may contribute to discrimination of PD patients and healthy controls,[10,11] and average genetic risk may be higher in patients with an early disease age at onset.[12] However, the clinical usefulness of these variants in prospectively predicting PD remains

untested. Also, it is unclear whether these risk variants evoke symptoms related to PD in individuals without clinical parkinsonism, leading to subtle problems in daily functioning.

We hypothesized that a genetic risk score based on currently identified risk loci would be a risk factor for incident PD in the general population, and that the genetic risk score would improve prediction of PD. Furthermore, we hypothesized that PD genes affect daily activities in community-dwelling individuals without parkinsonism.

METHODS

Study design and setting

The study was embedded in the Rotterdam Study, a large, prospective, population-based study in the Netherlands.[13,14] The original study cohort (RS-I) started in 1990 and consisted of 7,983 community-dwelling people aged 55 years and older, residing in the suburb Ommoord, Rotterdam. They were re-examined every 4 years, with the last re-examination between 2009 and 2011. In 2000, the cohort was expanded with 3,011 people aged 55 years and older (RS-II). The last follow-up examination for this subcohort took place between 2011 and 2012.

For PD prediction analyses, all participants in RS-I and RS-II free of parkinsonism and dementia at baseline with available genotype information on 26 risk loci for PD were eligible (n=7705). Of these individuals, 7,224 were interviewed at baseline on their smoking habits (never, past, current) and parental history of PD. Finally, 51 individuals refused to provide informed consent, leaving 7,167 participants (93.1%) for PD prediction analyses. We followed participants for a

maximum of twenty years for onset of PD from baseline until the first of: onset of parkinsonism, onset of dementia, death or 1 January 2011.

For basic activities of daily living (BADL) analyses, we invited all participants (n=3855) who were still alive, free of parkinsonism as well as free of dementia at the time of the last center visit round of both cohorts (RS-I in 2009-2011 and RS-II in 2011-2012). Of these individuals, 3,046 (79.0%) agreed to participate and were able to participate. Twenty-five individuals were excluded because of unknown smoking status at time of the BADL assessment and another twenty-four individuals did not complete their BADL assessment, leaving 2,997 individuals for BADL analyses.

Genotyping

The Illumina 550K (RSI), 550K duo, and 610 quad (RSII) arrays were used for genotyping. We removed samples with call rate below 97.5%, gender mismatch, excess autosomal heterozygosity, duplicates or family relations and ethnic outliers, and variants with call rate below 95.0%, failing missingness test, Hardy–Weinberg equilibrium p-value$<10^{-6}$, and minor allele frequency<1%. Genotypes were imputed using MACH/minimac software to the 1000 Genomes phase I version 3 reference panel (all population).

In the largest genome-wide association study of PD to date, 22 genome-wide significant primary variants, four secondary signals that remained significant in conditional analyses as well as two sub-genome-wide significant, potential risk variants were associated with the risk of disease at genome-wide significance in individuals without known mutations in genes associated with mendelian forms of

PD.[15] An overview of the of risk alleles as well as their reported effect size for the association with PD is presented in *Online Supplementary file 1*. Two of these variants were not genotyped in our dataset, nor reliably imputed (R2<.3), and also lacked a proxy variant (rs113579895, MAPT; rs115462410, HLA-DQB1), leaving 26 variants for analysis.

Ascertainment of parkinsonism and PD

A detailed description of assessment methods is provided in **Chapter 4.1**. In short, we used four overlapping modalities to screen for potential parkinsonism: in-person examinations (on average every 4 years), in-person interviews, use of antiparkinson medication, and clinical monitoring alerts.

Of all individuals who screened positive in any of these methods, complete medical records (including letters from medical records of specialists and general practitioners) were studied and case reports were drawn up covering all potentially relevant information to establish presence and cause of parkinsonism. These case reports were evaluated by a panel led by an experienced neurologist.

Parkinsonism was defined by presence of hypo- or bradykinesia in combination with ≥1 cardinal sign (resting tremor, rigidity or postural imbalance) observed by any physician, or a clinical diagnosis of parkinsonism by a neurologist or geriatrician (if motor examination details were unavailable). PD was only diagnosed after exclusion of secondary causes, in individuals with at least one of: 1) a clinical PD diagnosis by a neurologist or geriatrician; 2) positive response to dopaminergic treatment. Individuals with parkinsonism who did not fulfill PD criteria were considered secondary parkinsonism cases. After initial diagnosis,

medical records of all incident parkinsonism cases continued to be scrutinized until the end of the study period for new information that could lead to a revision of the diagnosis.

Ascertainment of dementia

Participants were screened for dementia at baseline and follow-up examinations using a three-step protocol,[16] comprising two brief tests of cognition to screen all individuals and the Cambridge Examination for Mental Disorders of the Elderly in individuals with positive screen results.[17] Additional information was obtained from in-person examination by a neuropsychologist, clinical monitoring and neuro-imaging . A consensus panel, led by a neurologist, decided on the final diagnosis in accordance with standard criteria using the DSM-III-R criteria for dementia.

Basic activities of daily living

Basic activities of daily living (BADL) was assessed based on the disability index from the Stanford Health Assessment Questionnaire, which consisted of 20 items constituting eight components: dressing and grooming, arising, eating, walking, hygiene, grip, reach, and activities.[18] In our study, two out of three items of eating (ability to lift a glass of milk and ability to cut meat) were combined into one. Items were scored from 0 to 3, as follows: 0=without difficulty, 1=with some difficulty, 2=with much difficulty, and 3=unable to. Component scores were calculated as the highest scored item per component.[18] The BADL score was calculated by summing all components, obtaining a score between 0 and 24. We

considered scores from 0 to 8 as no to mild disability and from 8 to 24 as moderate to severe disability.[19]

Statistical analysis

We constructed a genetic risk score for each individual, by adding up their number of risk alleles weighted by the log-transformed, reported risk-increasing or risk-decreasing effect size for the association with PD.[9] Risk scores were transformed into z-scores to facilitate evaluation of their effect per standard deviation increase. A higher genetic risk score corresponds to a larger weighted number of risk alleles and thus a higher risk of PD. We constructed two models: model I comprised age and sex for overall analyses, and only age for sex-stratified analyses. Model II comprised model I plus parental history of PD and smoking (never, past, current), and model III comprised model II plus the genetic risk score.

We investigated the association between the genetic risk score and incident PD by comparing each model using the method proposed by Fine and Gray, which takes into account the risk of competitive events (i.e., incident dementia or death).[20] In subanalyses, we separately added interaction terms between the genetic risk score and age, sex, smoking, and parental history to model III. The discriminative value of both models was expressed with Uno's C-statistic, which takes into account right-censoring.[15] Separately, we repeated the prediction analysis after addition of the *GBA* p.E326K variant to the risk score (its weight was calculated using the previously meta-analysed odds ratio of 1.71).[21] In other sensitivity analyses, we assessed the cross-sectional discriminative value of the risk score by combining prevalent PD cases with complete covariate data (n=68) and incident PD cases and performing logistic regression analyses.

To study the association between the genetic risk score and activities of daily living, we dichotomized BADL scores for having any difficulty in daily functioning or none. Because of the highly skewed distribution of BADL scores in our population,(*Supplementray file 2)* we used a binary logistic regression model to analyze the association of the genetic risk score with any difficulty in BADL, adjusting for age, sex and smoking. We report p-values based on 1,000 permutations. In separate subanalyses, we added interaction terms between the genetic risk score and age, sex and smoking to the model. Furthermore, we used multinomial logistic regression models to examine the association of the genetic score with mild and moderate to severe BADL impairment separately. Also, we examined associations between the genetic risk score and impairment on each BADL domain separately using logistic regression models. Finally, we examined the association of each of the 26 single risk variants with any impairment in BADL, adjusting for age, sex, and smoking with a Bonferroni correction for 26 comparisons (p=0.05/26).

RESULTS

Characteristics of the study population at risk of PD and the individuals examined for daily activities are presented in *Table 1*. In *Online Supplementary file 3*, we present population characteristics stratified by incident PD case status. During follow-up (median 12.1 years), 99 (1.4%) individuals suffered from incident PD and 930 (13.0%) from incident dementia, while a total of 3,286 (45.8%) individuals died.

Table 1 | Study population characteristics.

Characteristic	At risk of PD*	BADL examination**
Number of individuals	7,167	2,997
Women (%)	4,135 (57.7)	1,756 (58.6)
Age at baseline, mean, y (SD)	67.3 (8.4)	76.8 (6.6)
Smoking (%)		
Never	2,353 (32.8)	1,036 (34.6)
Past	3,237 (45.1)	1,672 (55.8)
Current	1,580 (22.0)	289 (9.6)
Parental history (%)		
No	6,962 (97.1)	-
1 parent with PD	205 (2.9)	-
2 parents with PD	3 (<0.1)	-

*PD, Parkinson's Disease; BADL, activities of daily living; y, year; SD, standard deviation. Smoking status was assessed at baseline for PD risk prediction analyses and during the last center visit for BADL analyses. *Included in longitudinal association and prediction analyses for Parkinson's Disease. **Included in cross-sectional association analyses for activities of daily living.*

In *Table 2*, we show that the genetic risk score was independently associated with the onset of PD. There was no significant interaction term between the genetic risk score and any of the covariates in the model (p=0.57 for interaction term with age; p=0.81 with sex; p=0.59 with smoking, p=0.88 with family history). Adding smoking and parental history to age and sex yielded borderline improvement in the prediction of incident PD (change in C=0.027 [-0.002; 0.056]), while addition of the genetic risk score to age and sex also produced improvement (change in C=0.038 [0.000; 0.076]). As shown in *Table 2*, the genetic risk score did not improve prediction beyond age, sex, smoking and parental history (change in C =0.011 [-0.011; 0.033]). The *GBA* p.E326K variant had a minor allele frequency of 0.021 in our population, and incorporation of this variant in the genetic risk score did not affect its incremental predictive value (change in C = 0.009 [-0.009;0.026]).

Table 2 | Prediction of incident Parkinson's Disease in the general population.

	HR (95% CI)	C-statistic (95% CI)
Model I		0.659 (0.599; 0.720)
Age	1.05 (1.03; 1.07)	
Female	0.66 (0.44; 0.98)	
Model II		0.687 (0.628; 0.745)
Age	1.05 (1.03; 1.07)	
Female	0.48 (0.30; 0.76)	
Smoking (past)	0.57 (0.35; 0.94)	
Smoking (current)	0.36 (0.18; 0.70)	
≥ 1 parent with PD	1.29 (0.40; 4.15)	
Model III		0.698 (0.635; 0.760)
Age	1.05 (1.02; 1.07)	
Female	0.48 (0.30; 0.76)	
Smoking (past)	0.57 (0.35; 0.93)	
Smoking (current)	0.36 (0.19; 0.71)	
≥ 1 parent with PD	1.25 (0.39; 4.03)	
Genetic risk score	1.25 (1.02; 1.55)	

HR, hazard ratio for incident Parkinson's Disease per standard deviation increase in risk score. CI, confidence interval. For smoking, reference category was never.

The univariate C-statistic of the genetic risk score was 0.56 [0.48; 0.64]. In cross-sectional sensitivity analyses, the genetic risk score yielded a similarly small improvement of C-statistics beyond age, sex, smoking and parental history (C=0.663 to C=0.677).

The genetic risk score was associated with any impairment in BADL (p=0.016). There was no significant interaction terms of the genetic risk score with age, sex, or smoking (p>0.10 for all interaction terms). As shown in *Table 3*, the genetic risk score was significantly associated with mild impairment (p=0.020), but not with

moderate to severe impairment (p=0.768) in separate analyses. In contrast to the overall BADL-score, the genetic risk score was not associated with any of the eight BADL domains separately (p>0.20 for each domain). None of the 26 single risk variants was associated with impairment in BADL after Bonferroni correction. Interestingly, risk alleles in three PD loci were nominally borderline associated with any impairment BADL: *GCH1* (rs11158026; p=0.055), *CCDC62* (rs11060180; p=0.058) and *GBA-SYT11* (rs35749011; p=0.054). None of the remaining 23 variants was associated with any impairment in BADL (p>0.10 for each variant).

Table 3 | Genetic risk score and basic activities of daily living.

BADL	N (%)	OR (95% CI)	P value
No impairment	461 (15.4)	(reference)	
Any impairment	2536 (84.6)	1.110 (1.002; 1.230)	0.016
Mild impairment	2017 (67.3)	1.123 (1.013; 1.246)	0.020
Moderate to severe impairment	519 (17.3)	1.020 (0.889; 1.171)	0.768

BADL, basic activities of daily living. N, number of individuals. OR, odds ratio. 95% CI, 95% confidence interval. Odds ratio per standard deviation increase in genetic risk score. Reference category for both mild and moderate to severe impairment is no impairment. Analyses were adjusted for age, sex and smoking.

DISCUSSION

In this large population-based sample with a median of 12 years of follow-up, we found that a genetic risk score for PD based on the most recent set of genome-wide significant variants was associated with a modest but significant increase in the risk of PD. However, in addition to age, sex, smoking status at baseline and parental history, the genetic risk score hardly improved the prediction of incident

PD. In cross-sectional analyses, we further found that the genetic risk score was associated with any and with mild impairment in BADL.

As far as we know, only case–control studies have previously been employed to examine the use of a genetic risk score for PD to discriminate between PD cases and healthy controls.[10-12,22] These studies showed that a risk score based on these variants may contribute to discrimination of PD patients and healthy controls,[10,11] and average genetic risk may be higher in patients with an early disease age at onset.[12] In a recent diagnostic case-control study of PD, the univariate C-statistic of a genetic risk score that comprised 30 genetic variants including the 26 used in our study ranged from 0.62 to 0.64,[11] which was slightly higher than in our predictive study (C-statistic=0.56). This relatively small difference may be explained by the difference in study design: in case-control studies, controls are recruited with strict criteria that ensure maximal distinction from PD cases, whereas participants in prospective, population-based studies such as the Rotterdam Study are included irrespective of PD risk. The advantage of prospective population-based studies is that all participants were included and followed up using the same methodology, and following up individuals in the general population presumably ensured a realistic estimate of the risk of incident PD. Several limitations of our study should be noted, however. We lacked histologic confirmation of specific pathologies, suggesting that some misclassification of PD occurred in our study. The detailed in-person and clinical information on the presence and possible causes of parkinsonism throughout the study period make it unlikely that the misclassification was differential (e.g. that we systematically misclassified essential tremor patients as PD cases). Still, non-differential misclassification may have caused us to underestimate the predictive ability of the genetic risk score for histologically confirmed PD. Also, we note that

part of the RS-I cohort used for prediction of PD was also among the discovery cohorts of the PD genes: the overlap comprised 44 incident PD cases (0.3%) and 5,609 controls (5.9%).[9] We believe that it is unlikely that this small proportion of overlap influenced our current findings. In addition, current effect estimates were based on a GWAS meta-analysis of PD cases across a wide variety of Caucasian populations. It is possible that other variants have larger effects in the Dutch population than the tagging SNPs published in the meta-analysis. Including these population-specific variants in the risk score could improve power. Furthermore, we were probably underpowered to a small improvement in PD prediction and, similarly, to detect effect modification of the association of the genetic risk score by traditional risk factors and parental history. In addition, we could only assess the predictive value of the risk score for incident PD in individuals aged 55 years and older. Since high polygenic risk is associated with a lower age of onset of PD,[12] this probably led to a slight underestimate of the predictive value of the genetic risk score, considering the relatively small proportion of PD patients aged younger than 55 at a population level.[1]

The main motivation for learning how to predict PD is to identify PD patients as early as possible. At this time, although neuroprotective agents with sustainable effects remain elusive, PD manifestations can often be treated or delayed effectively, and surveillance could allow early symptomatic treatments, perhaps with long-term benefits on quality of life.[23,24] As the pathological processes of PD advance, early clinical features become increasingly more prevalent in prediagnostic PD patients than in controls,[25] and discrimination of clinical PD patients and healthy controls can be accurately established (as reflected by high C-statistics) using just one early feature (impaired olfaction).[11] However, during the early pathological phase of PD, clinical differences between prediagnostic PD

patients and controls are generally not yet overt, and discrimination between these groups is less accurate, as reflected by lower C-statistics. Early prediction is therefore based on basic demographics (e.g., age, sex, family history) and environmental risk factors (e.g., smoking, exposure to pesticides). The discriminative value of demographics is remarkably similar for long-term prediction (as in our study) and clinical diagnosis of PD as in diagnostic studies,[10,11] with integrative demographic C-statistics typically ranging from 0.60 to 0.70. Smoking was previously included in a diagnostic model for PD,[10] but contributed insufficient independent information to be included in a recent integrative diagnostic algorithm for PD.[11] The latter was surprising for two reasons. First, smoking is common at a population level, and current smoking in particular is strongly inversely associated with PD in case-controls studies.[26] Second, PD patients who do smoke are able to quit smoking more easily than controls,[27] which would theoretically make the discriminative value of smoking even higher for PD diagnosis than for PD prediction.

Over the past few years, genetic risk scores have been shown to be of marginal value in prediction of diseases with strong preexistent demographic and clinical factor-based predictive models.[28,29] However, they have enabled improvement in prediction of diseases without such models,[30,31] and in a recent diagnostic study of Alzheimer's Disease, genetic risk scores based on GWAS variants and *APOE* variants improved diagnostic accuracy beyond age and sex.[32] In this study of more than 7,000 individuals, we showed that addition of a genetic risk score for PD did not improve prediction beyond age, sex, smoking and parental history. Thus, our findings do not support a role for routine PD risk allele genotyping in a clinical setting at this time. This is similar to our previous observation of that genetic risk variants had limited predictive value for Alzheimer's Disease and all-

cause dementia.[33,34] As more PD risk variants become known, however, their incorporation into the genetic risk score may explain more of the heritability that was first implied by familial aggregation of PD,[35] and is now estimated to be 0.27.[8] A recent meta-analysis showed that mild to severe GBA mutations are more common in PD populations than in controls.[6] For the carriers of the severe *GBA* mutations, it has been suggested that the high increase in risk of PD (OR 14.6 – 19.3) may warrant a closer clinical follow-up,[6] similar to carriers of the G2019S mutation in the *LRRK2* gene.[36] However, the predictive value of such rare variants at a population level remains undetermined, and we note that the current genetic risk score did not include the G2019S mutation in *LRKK2* and only focused on the p.E326K variant in *GBA*.

To our knowledge, this is the first study to investigate the relationship of a genetic risk score for PD with daily activities in the general population. The genetic risk score was associated with any impairment in BADL, suggesting that alleles with an established association with PD may also affect prodromal phenotypes linked with PD in the general PD-free population. Interestingly, we observed a clear association of the genetic risk score with mild impairment in BADL, but not with moderate to severe impairment. We offer two possible explanations for this observation. First, since we excluded individuals with parkinsonism and dementia from our analyses, the majority of individuals with moderate to severe impairment probably comprised individuals with common, non-neurodegenerative diseases (e.g., locomotor diseases, COPD [37]). We note that we are unaware of substantial genetic overlap with PD for these diseases or of empirical evidence for antagonistic pleiotropic effects of PD risk variants on BADL. Second, we studied risk variants that are relatively common in the general

population, and these variants may affect BADL more subtly than rarer risk variants with larger effect sizes on the risk of PD.

In conclusion, in this study in the general population, a genetic risk score based on 26 independent risk variants was associated with a higher risk of incident PD and a larger probability of impairment in BADL, but did not result in a substantially better prediction of PD beyond age, sex, smoking and parental history. Our results suggest that the use of this weighted combination of known risk loci is not yet as useful for the prediction of the risk of PD as it is for further elucidating the etiology of the disease. However, we were probably underpowered to detect a small improvement in PD prediction.

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Chapter 5.2

Novel MicroRNA-related variants

Chapter 5.2

ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNAs that serve as key regulators of gene expression. MiRNAs have been shown to be involved in a wide-range of biological processes including neurodegenerative diseases. Genetic variants in miRNAs and in miRNA-binding sites on their target genes could affect miRNA function and contribute to disease risk. We investigated the association of miRNA-related genetic variants with Parkinson's Disease (PD) using data from the largest available GWAS on PD. Out of 243 variants in miRNAs, we identified rs897984:T>C in miR-4519 (p-value= 1.3×10^{-5}, OR= 0.93) and rs11651671:A>G in miR-548at-5p (p-value= 1.1×10^{-6}, OR= 1.09) to be associated with PD. We showed that the variant's mutant alleles change the secondary structure and decrease expression level of their related miRNAs. Subsequently, we highlighted target genes that might mediate the effects of miR-4519 and miR-548at-5p on PD. Among them, we experimentally showed that *NSF* is a direct target of miR-4519. Furthermore, among 48,844 variants in miRNA-binding sites, we found 32 variants (in 13 genes) that are associated with PD. Four of the host genes, *CTSB*, *STX1B*, *IGSF9B* and *HSD3B7*, had not previously been reported to be associated with PD. We provide evidence supporting the potential impact of the identified miRNA binding site-variants on miRNA-mediated regulation of their host genes. Our findings support the idea that miRNAs play a role in PD and highlight a number of variants in miRNA-related sequences that may affect miRNA-mediated regulation of PD genes.

BACKGROUND

Neurodegenerative diseases collectively represent one of the major worldwide causes of morbidity and healthcare costs to society.[1] Parkinson disease (PD) is the second most common neurodegenerative disorder.[2] and its prevalence and burden at the population level are projected to grow dramatically as the size of elderly populations increases.[3] Clinically, PD is characterized by a combination of motor symptoms, known as parkinsonism, and a range of non-motor symptoms, such as cognitive decline and autonomic dysfunction, that contribute to a devastating loss of quality of life.[4,5] PD is thought to be a complex disease, and genetic factors have a substantial impact on the phenotypic variation of the disease.[6] Over the past few decades, enormous efforts have been done to discover genetic factors that play a role in development of PD. In recent years, the large-scale genome-wide association studies (GWAS) have enabled the discovery of hundreds genetic variants that are associated with PD risk.[7,8] Nevertheless, most of the identified variants are mapped to non-coding regions of the genome, and their causal mechanisms remain to be investigated.

MicroRNAs (miRNA) are a class of small non-coding RNAs, that post-transcriptionally regulate gene expression.[9,10] MiRNAs have been shown to be involved in a wide range of biological processes and human diseases including neurodegenerative disorders.[11-13] In addition, a number of dysregulated miRNAs have been reported to be associated with PD in patients and animal models.[14-16] The regulatory functions of miRNAs are accomplished through binding of the nucleotides 2-8 from their 5'end (the seed region) to the complementary sequences at the target mRNAs, resulting in repression of translation or a decreased stability of target mRNA.[9,10] Genetic variants that fall within miRNA-related sequences may affect miRNA function and due to aberrant target genes

expression, the variants could modify susceptibility to disease.[17] Recently, we and others have been able to show a number of polymorphisms in miRNAs or their target genes that may contribute to phenotypic variations.[18-22] However, no systematic investigation of the impact of such variants on the risk of PD has been published to date.

In this study, we examined the association of variants in miRNAs as well as miRNA binding sites with PD using data from the largest GWAS. We subsequently integrated our GWAS findings with computational and biological information (such miRNA and gene expression profiles) and performed experimental studies to provide evidence for functionality of the identified miRNA-related variants associated with PD.

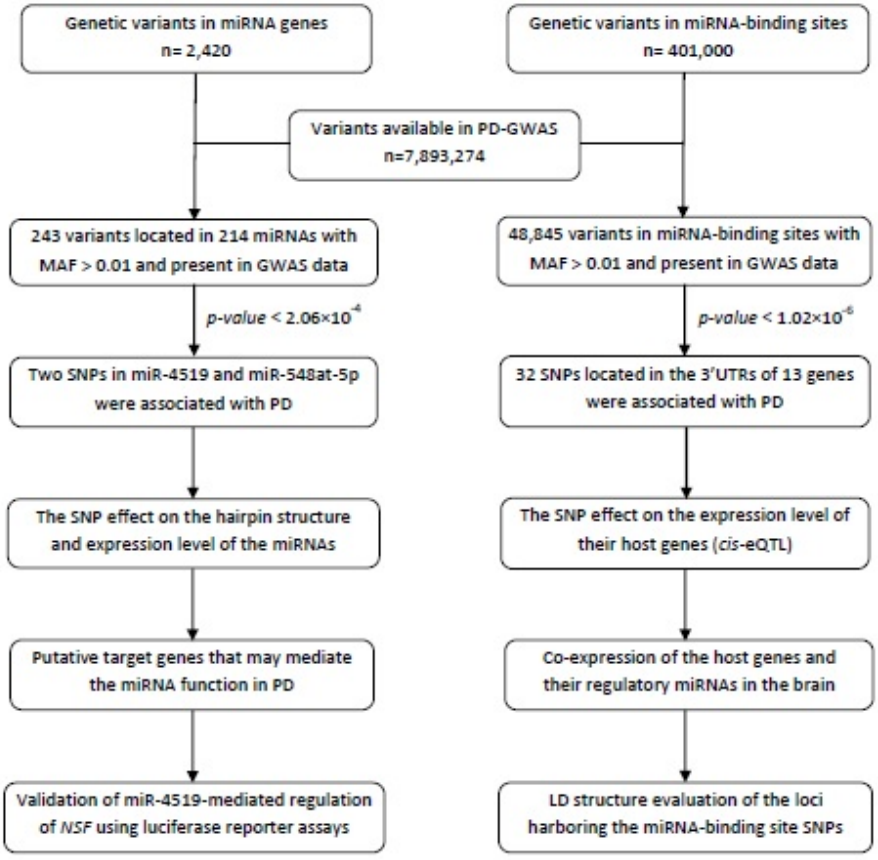
METHODS

Identification of genetic variants in miRNAs and miRNA-binding sites

We retrieved all genetic variants that are localized in miRNAs or miRNA binding sites on their target genes by reviewing the literature and using two online databases: miRNASNP (v2.0)[23] and PolymiRTS (v3.0)[24]. We retained a total of 2,420 variants in all human precursor (60-80 nt) and mature (20-24 nt) miRNA sequences. We excluded variants with minor allele frequency (MAF) < 0.01. Of the remaining variants, we included 243 single-nucleotide polymorphisms (SNPs) in 214 miRNAs that were present in the recent GWAS of PD.[8] Furthermore, we retained around 401,000 miRNA binding site-variants that were predicted to affect the match to the seed region of miRNAs. Of these, 45,631 SNPs with MAF > 0.01 and present in the

GWAS of PD were included.[8] A flowchart of our approach to retrieve the variants located in miRNAs and miRNA binding sites is shown in *Figure 1*.

Figure 1 | approach to identify genetic variants in miRNAs and miRNA-binding sites that are associated with Parkinson's Disease.



MAF, minor allele frequency; LD, linkage disequilibrium.

Genome-wide association study on PD

We examined the association of retrieved variants in miRNAs and miRNA-binding sites with PD using summary statistics data from the largest GWAS on PD across

13,708 PD cases (39% female) and 95,282 controls (46% female).[8] The GWAS data were imputed to 1000 Genomes project reference panel, providing data for 7,893,274 variants. Details about the consortium and participants are described elsewhere.[8] We used the Bonferroni correction to adjust *p*-value for the number of tests and significant threshold was set at 2.1×10^{-4} (0.05 / 243) for variants in miRNAs and 1.1×10^{-6} (0.05 / 45,631) for variants in miRNA binding sites.

Analyzing the variant effect on miRNA structure and expression

For miRNA variants that were associated with PD, we used the Vienna RNAfold algorithm (ViennaRNA package 2.0) to predict the variant effect on the hairpin structure of miRNA.[25] Difference in minimum free energy (MFE) of the thermodynamic ensemble of precursor miRNA (pre-miRNA) sequence containing the mutant versus the wild type allele may indicate an altered miRNA processing. Furthermore, to experimentally examine the variant's effect on the expression level of mature miRNA, we cloned the pre-miRNA sequences containing either the wild type or mutant allele behind the gene encoding green fluorescent protein (GFP) in the expression plasmid MSCV-BC, resulting in GFP-miRNA fusion transcripts.[26] The inserts of all constructs were validated by Sanger sequencing. HEK293 cell transfection, total RNA isolation and quantitative PCRs were performed as previously described.[26] The primers are shown in *Online Supplementary Table S1*. The experiment was performed in triplicate.

quantitative-PCR of miRNAs

We examined whether miRNAs hosting the variants associated with PD are expressed in the human brain. To this end, brain tissue was obtained from the Netherlands Brain Bank (Amsterdam, The Netherlands). All samples were free of neurological disease. For isolation of total RNA, five cryopreserved sections of 40 µm were homogenized in 250 µl Trizol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was isolated from 6 brain samples (3 white matter and 3 gray matter). The concentration and purity of RNA samples were determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop, Wilmington, DE). The expression levels of miRNAs were determined with TaqMan MicroRNA Assays according to manufacturer's protocols (Applied Biosystems, Foster City, CA, USA). RNU6B was used as an endogenous control. All experiments were performed in triplicate.

Association of miRNA target genes with PD

For miRNAs that were associated with PD, we further examined which of the target genes may mediate the effect of miRNAs on PD. To do this, we extracted all predicted target genes of the identified miRNAs using two online databases, TargetScan v7.0 (http://www.targetscan.org)[27] and miRDB (http://mirdb.org/miRDB)[28]. The miRNA target genes that are listed in both databases were used for our analyses. Next, we used the PD-GWAS data in a candidate gene approach to identify those target genes that are likely to be involved in developing PD. We retrieved the summary statistics for the association of all genetic variants in the target genes with PD. The significance threshold for this analysis was set using the Bonferroni correction based on the number of studied variants. Additionally, we performed Ingenuity Pathway Analysis (IPA) to explore the pathways in which target genes of the identified miRNAs may play a

role (http://www.ingenuity.com/products/ipa). A list of all putative target genes of each miRNA were uploaded and a core IPA analysis were performed using the default settings. We mapped the miRNA target genes to biological functions or canonical pathways to determine whether they are enriched in neurological networks. The *p*-values are calculated using the right-tailed Fisher Exact Test and a p-value less than 0.05 indicates a statistically significant, non-random association.

Luciferase reporter assay

Primers were designed to amplify the pre-miRNA included in the restriction enzyme sites XhoI for the forward primer and EcoRI for the reverse primer. The miRNA expression vector was cloned in the MSCV-BC vector. In addition, primers were designed to clone the 3'UTR sequence of target gene included in the restriction enzyme sites XbaI for the forward primer and ApaI for the reverse. The 3'UTR sequences of target gene (either wild-type or mutated), containing the putative binding site of miRNA, were cloned downstream of the Luciferase gene in the pGL3 vectors as previously described.[26] The primers are shown in *Online Supplementary Tables S1* and *S2*. The inserts of all constructs were validated by Sanger sequencing. COS cells were plated into 96-well plates and co-transfected with MSCV-miRNA, pGL3 containing the different 3'UTR and a plasmid expressing the Renilla transfection control. Luciferase activity was determined with the Dual-Glo Luciferase Assay System according to manufacturer's protocol (Promega). Renilla luciferase activity was used for normalization. All experiments were performed five times.

Expression quantitative trait loci (eQTLs)

We scanned cis-eQTL data to examine the association between miRNA binding site variants and the related transcript expression levels. We used two online web browsers: Genenetwork (http://genenetwork.nl/bloodeqtlbrowser/) and GTEx V4 (http://www.broadinstitute.org/gtex/). The GTEx platform provides information on eQTL in different tissues including brain. In addition, we used the eQTL data in whole blood from the Gene network because of a very large sample size (n=5,311). The designs of these studies have been described in detail elsewhere.[29,30]

Expression of the identified target genes and related miRNAs in relevant tissues

To search for expression of the identified target genes and regulatory miRNAs in relevant tissues, we employed several web tools. The Illumina's Human Body Map 2.0 data (http:// www.ensembl.info/blog/2011/05/24/human-bodymap-2-0-data-from-illumina/) were used to examine the expression of the genes hosting miRNA binding site-variants across different tissues. This database provides RNASeq data of 16 human tissue types, including brain. To scan the expression of related miRNAs in the brain, we used the Human MiRNA Expression Database (HMED),[31] mimiRNA[32] and PhenomiR[33] databases. We further searched the literature via PubMed using the search terms of miRNA name and "expression" for those not implicated in the listed databases.

Chapter 5.2

Analyzing functional characteristics of the identified miRNA binding site-variants

We evaluated LD blocks of the identified miRNA binding site variants to examine whether there are other SNPs in high LD in the related loci that may drive the observed GWAS associations. To this end, the list of PD-associated variants in miRNA binding sites were submitted to the SNAP web tool (http://www.broadinstitute.org/mpg/snap/id) using R^2 threshold > 0.8, limit distance 500 kb, and population panel CEU to retrieve their proxy SNPs in the 1000 G. We then utilized the HaploReg web tool v3 (http://www.broadinstitute.org/ mammals/haploreg/haploreg_v3.php) to predict the effect of SNPs on protein structure, gene regulation, and splicing. Other information, including miRNA sequences, miRNA host genes and miRNA conservation in different species was obtained from TargetScan v7.0 and miRBase (release 20)[34] databases.

RESULTS

Two miRNA variants were associated with PD

We studied the association of 243 SNPs (with MAF> 0.01) located in 214 miRNAs with PD. Of these, rs11651671 (Chr17:42494785,A>G) in miR-548at-5p (*p*-value=1.06×10^{-6} and OR=1.09) and rs897984 (Chr16:30875322, T>C) in miR-4519 (*p*-value=1.34×10^{-5} and OR=0.93) were significantly (*p*-value < 2.1×10^{-4}) associated with PD. *Online Supplementary Table S3* shows all miRNA variants that are associated with PD with a *p*-value <0.05. The forest association plots showing meta-analysis of the association of these two miRNA variants with PD are shown in *Online Supplementary Figure S1*.

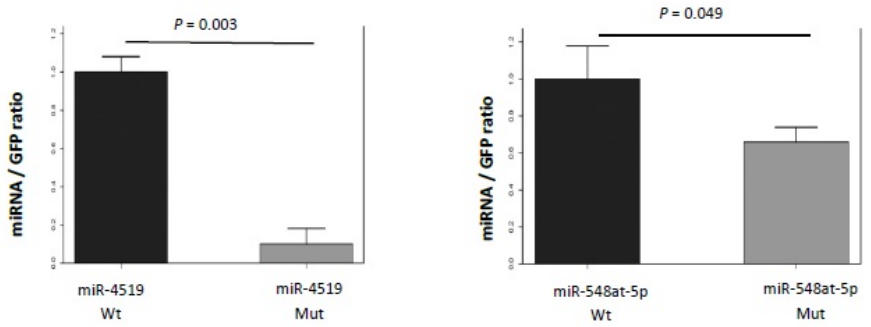
The effect of variants on miR-4519 and miR-548at-5p structure and expression

We generated the hairpin structures of miR-4519 and miR-548at-5p containing the wild type or mutant alleles using the Vienna RNAfold algorithm[25]. We noted a +6.1 kcal/mol difference in the minimum free energy of the predicted thermodynamic ensemble of the mutant versus the wild type miR-548at-5p structure (*Online Supplementary Figure S2*), which may affect the processing of pre-miRNA. The predicted change in minimum free energy of the thermodynamic ensemble of the hairpin structure of miR-4519 containing the mutant versus the wild type was -0.3 kcal/mol (*Online Supplementary Table S3*). We then examined the expression levels of these miRNAs from two instances: the wild type pre-miRNA and the mutant pre-miRNA. We cloned the pre-miRNAs sequences (containing either wild-type allele or mutant allele) behind the GFP in the expression plasmid. Transient transfection of the miRNAs in HEK293 cells showed significantly reduced levels of the mature miRNAs from the mutant alleles relative to GFP compared with the wild-type alleles, where the rs897984 mutant allele reduced the expression level of miR-4519 by 90% (*p*-value=0.003) and the rs11651671 mutant allele decreased the expression level of miR-548at-5p by 30% (*p*-value=0.049) (*Figure 2*).

Association of miR-4519 and miR-548at-5p target genes with PD

MiRNAs act through regulation of their target gene expression. We thus assessed whether 342 putative target genes of miR-4519 and 676 putative target genes of miR-548at-5p are implicated in neurological pathways using IPA. This analysis showed several target genes of miR-4519 to be (in)directly linked with Nervous System Development and Function Networks.(*Online Supplementary Table S4*)

Figure 2 | Impact of rs897984 in miR-4519 and rs11651671 in miR-548at-5p on expression levels of mature miRNAs.



As shown, the levels of miR-4519 and miR-548at-5p are significantly reduced from the mutant constructs (minor allele) relative to GFP compared to the wild-type constructs.

We then examined the association of genetic variants in all putative target genes of miR-4519 and miR-548at-5p with PD using GWAS data. We studied 76,457 SNPs in 342 target genes of miR-4519, with the significant threshold of 6.5×10^{-7}, and found four target genes, *NSF*, *TMEM163*, *CCNT2* and *SH3GL2,* to be associated with PD (*Table 1*). For miR-548at-5p, we assessed 153,018 SNPs in 676 target genes, with the significance threshold of 3.3×10^{-7}, and identified *GCH1*, *MMRN1*, *CCNT2* and *DCUN1D1* to be associated with PD (*Table 1*).

Table 1 | Association of two variants in miR-4519 and miR-548at-5p with Parkinson's Disease.

miRNA ID	SNP ID (A1/A2)	OR GWAS	*p*-value GWAS	PD-associated target genes (*p*-value)
miR-4519	rs897984 (G/A)	0.93	1.3×10^{-5}	*NSF* (3.9×10^{-29}) ; *TMEM163* (2.7×10^{-13}) ; *CCNT2* (8.6×10^{-9}) ; *SH3GL2* (1.3×10^{-7})
miR-548at-5p	rs11651671 (A/G)	1.09	1.1×10^{-6}	*MMRN1* (4.4×10^{-15}) ; *GCH1* (2.0×10^{-10}) ; *CCNT2* (8.6×10^{-9}) ; *DCUN1D1* (8.7×10^{-8})

A1, Allele 1; A2, Allele 2; OR, Odds ratio; GWAS, Genome-wide association study of PD (Nalls et al., 2014); The p-value for the highlighted target genes is p-value of the SNP in the gene with the most significant association with PD.

Using the Human Body Map 2.0 data, we showed that the highlighted target genes of miR-4519 and miR-548at-5p are expressed in the brain (*Online Supplementary Table S5*). Next, we asked whether the identified miRNAs are expressed in the brain. Our data showed that miR-4519 is expressed at detectable levels, the Ct-value range between 31.5 and 34.1, in both white and gray matter of the human brain (*Online Supplementary Table S6*).

Subsequently, we examined whether miR-4519 control the expression level of its top identified target gene, *NSF*, in-vitro. We generated expression vector containing the pre-miR-4519 sequence and co-transfected the construct with Luciferase reporters containing the wild-type and mutant 3′ UTR of *NSF*. We found that expression of miR-4519 significantly decreases the Luciferase activity of wild type *NSF* reporter, compared to the mutated *NSF* reporter, *p*-value= 0.0002 These data indicate that *NSF* is a direct target of miR-4519.

Multiple miRNA binding site-variants were associated with PD

The associations of 45,631 miRNA binding site-variants with PD are shown in a Manhattan plot (*Figure 3*). Of these, we found 32 SNPs located in the 3′UTR of 13 genes that are significantly associated with PD (*p*-value $<1.1\times10^{-6}$) (*Table 2*). These SNPs are predicted to affect miRNA-mediated regulation of their host genes by disrupting, creating or modifying a number of miRNA binding sites that are depicted in *Online Supplementary Tables S7* and *S8*. Out of 13 genes hosting the 32 SNPs, the association of 9 genes with PD had already been reported by GWAS [8]. Four others that were not previously reported for PD include *HSD3B7* (*p*-value=5.2×10^{-8}), *IGSF9B* (*p*-value= 2.6×10^{-7}), *CSTB* (*p*-value=4.8×10^{-7}) and *STX1B* (*p*-value=1.2×10^{-7}).

Table 2 | miRNA binding site variants associated with Parkinson's Disease.

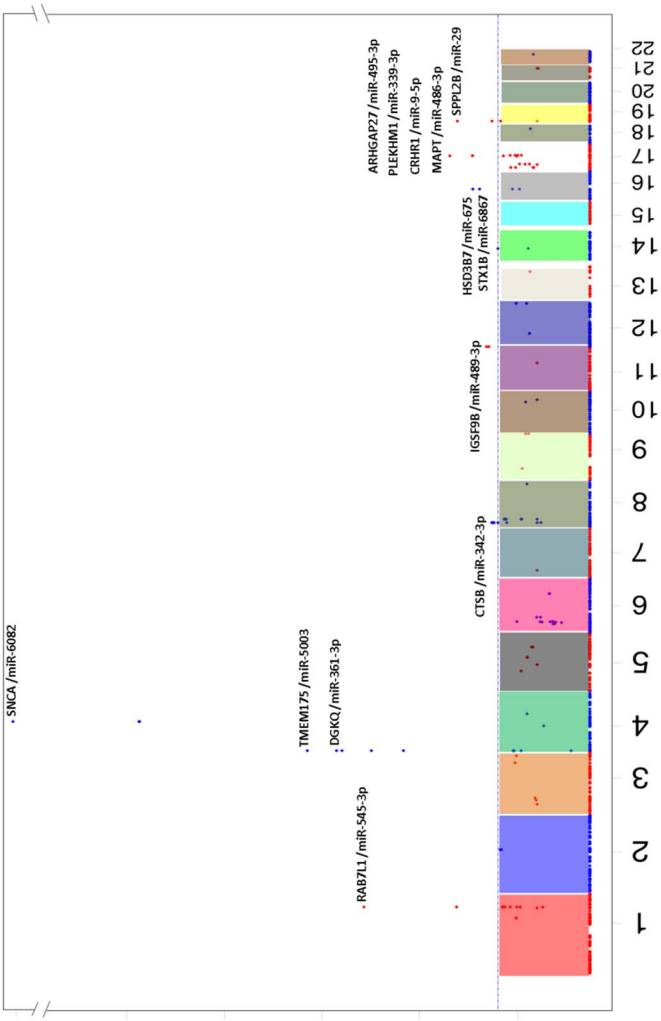
SNP ID	Chr: position	A1/A2	MAF	Gene	P-value GWAS	OR GWAS
rs4763	chr17:43471489	A/G	0.14	*ARHGAP27*	5.0×10^{-8}	<1
rs8327	chr17:43472507	G/A	0.24	*ARHGAP27*	3.5×10^{-9}	1.21
rs16940681	chr17:43912159	C/G	0.17	*CRHR1*	5.0×10^{-8}	<1
rs4482334	chr17:43912830	T/C	0.22	*CRHR1*	5.0×10^{-8}	>1
rs4640231	chr17:43912786	C/G	0.22	*CRHR1*	5.0×10^{-8}	<1
rs878886	chr17:43912490	C/G	0.22	*CRHR1*	5.0×10^{-8}	>1
rs878888	chr17:43912635	A/G	0.22	*CRHR1*	5.0×10^{-8}	>1
rs2740592	chr8:11701253	A/G	0.21	*CTSB*	4.8×10^{-7}	0.91
rs2645425	chr8:11701278	A/G	0.21	*CTSB*	5.2×10^{-7}	0.91
rs4839	chr8:11701933	C/T	0.21	*CTSB*	6.0×10^{-7}	0.91
rs3947	chr8:11702375	A/G	0.21	*CTSB*	6.4×10^{-7}	0.91
rs4583705	chr4:973036	T/C	0.14	*DGKQ*	1.5×10^{-11}	1.24
rs3733349	chr4:954311	T/C	0.43	*DGKQ*	$3.4\times^{10-13}$	0.89
rs3733345	chr4:954247	T/G	0.45	*DGKQ*	1.0×10^{-14}	0.88
rs4690326	chr4:953698	C/A	0.45	*DGKQ*	5.4×10^{-15}	0.88
rs2305880	chr16:30999462	T/C	0.25	*HSD3B7*	5.2×10^{-8}	0.91
rs3802922	chr11:133786945	C/A	0.19	*IGSF9B*	2.6×10^{-7}	1.11
rs3802921	chr11:133786993	C/T	0.20	*IGSF9B*	2.7×10^{-7}	1.11
rs3802920	chr11:133787001	T/G	0.19	*IGSF9B*	3.4×10^{-7}	1.11
rs1052594	chr17:44102689	C/G	0.20	*MAPT*	5.0×10^{-8}	<1
rs11012	chr17:43513441	T/C	0.15	*PLEKHM1*	5.0×10^{-8}	<1
rs62064654	chr17:43513896	T/C	0.15	*PLEKHM1*	5.0×10^{-8}	<1
rs62064655	chr17:43514954	A/G	0.42	*PLEKHM1*	5.0×10^{-8}	<1
rs11557080	chr1:205737739	G/A	0.23	*RAB7L1*	7.6×10^{-9}	0.87
rs708723	chr1:205739266	C/T	0.44	*RAB7L1*	1.4×10^{-13}	0.89
rs356165	chr4:90646886	A/G	0.48	*SNCA*	2.9×10^{-62}	0.76
rs3857053	chr4:90645674	T/C	0.11	*SNCA*	4.3×10^{-25}	1.34
rs1045722	chr4:90645671	A/T	0.11	*SNCA*	4.6×10^{-25}	1.34
rs10420958	chr19:2353368	A/G	0.48	*SPPL2B*	4.9×10^{-7}	0.92
rs1128402	chr19:2353150	A/C	0.28	*SPPL2B*	8.4×10^{-9}	1.12
rs8060857	chr16:31002720	G/A	0.24	*STX1B*	1.2×10^{-7}	0.91
rs748483	chr4:952409	G/A	0.14	*TMEM175*	1.8×10^{-16}	1.22

Chr, chromosome; A1, ancestor allele; A2, alternative allele; MAF, Minor Allele Frequency in 1000G CEU; OR, Odds ratio; GWAS, Genome-wide association study.

Supporting evidence for potential functionality of the identified miRNA-binding site variants

We searched for cis-eQTL of 13 genes hosting the 32 identified miRNA binding site-variants and found 6 SNPs that are correlated with expression levels of their host genes. This includes rs10420958, which was associated with *SPPL2B* expression in the brain, and five SNPs (including rs708723 and rs2645425) that were associated with expression levels of *CTSB* and *RAB7L1* in blood. Using the HaploReg web tool, we searched for potential functionality of the 32 SNPs and their proxies in high LD on gene regulation. This analysis showed that some of the miRNA binding site SNPs have no proxy or only week proxies in the related loci, such as rs8327, rs356165, rs708723 and rs1128402 (*Table 3*). Furthermore, through scanning the expression of 13 host genes, we found that *SNCA*, *CTSB*, *MAPT* and *STX1B* are abundantly expressed in the brain, using the Human Body Map 2.0 data (*Online Supplementary Table S9*). We also found evidence for expression of several of the regulatory miRNAs in the brain, in particular miR-342-3p, miR-29a-5p, miR-9-5p, using the miRNA expression databases (*Online Supplementar S7*). A summary of the evidence we found suggesting the functisonality of some of the identified miRNA binding site-SNPs in their related loci are depicted in *Table 3*.

Figure 3 | The association of miRNA-binding site variants with Parkinson's Disease.



This Manhattan plot shows the association of miRNA-binding site variants (with a p-value < 0.05) with Parkinson's Disease. Dashed line indicates the significant study threshold (1.02 × 10−6).

Table 3 (part I) | Functional characteristics of the identified miRNA binding site-variants in the 3'UTR of 13 genes associated with Parkinson's Disease.

	rs8327	rs708723	rs356165	rs16940681	rs2645425	rs4690326	rs2305880
GWAS							
P value	3.5×10–9	1.4×10–13	2.9×10–62	5.0×10–8	5.2×10–7	5.4×10–15	5.2×10–8
OR	1.21	0.89	0.76	<1	0.91	0.88	0.91
Gene							
Host gene	ARHGAP27	RAB7L1	SNCA	CRHR1	CTSB	DGKQ	HSD3B7
Brain Exp	1.4	7.5	187	2.0	135	1.4	1.1
Proxy							
All SNPs	0	3	2	1150	35	4	56
Nonsynon	0	0	0	13	0	0	1
eQTL							
Blood	–	2.3×10–42	–	–	7.3×10–172	–	–
Brain	–	–	–	–	–	–	–
miR-binding site							
Dis (A1)	miR-495-3p	NC	miR-6082	miR-9-5p	None	NC	NC
ΔScore	-0.02	–	–0.12	NA	–	–	–
Cre (A2)	None	miR-545-3p	None	NC	miR-342-3p	miR-361-3p	miR-675-5p
ΔScore	–	–0.08	–	–	NA	–0.23	-0.48

Supporting evidence for 13 miRNA binding site-variants that are associated with PD. For genes hosting more than one variant in miRNA binding sites, a variant with a higher probability of being functional is reported in the table. In addition, for variants that are predicted to affect more than one miRNA, the highly conserved miRNA in each situation is mentioned (full information provided in the Supplementary data). miR, miRNA; OR, Odds ratio; Exp, Expression; Nonsynon, Non-Synonymous SNPs (using the HaploReg web tool v3); ΔScore, Context score change (using the PolymiRTS database v3); Dis, Disruption binding site; Cre, Creation binding site; A1, Allele 1; A2, Allele 2; None, No binding site for miRNAs; NC, Binding site for a non-conserved miRNA; NA, Not available. The value for eQTL is p-value; the value for brain expression is fragments per kb of exon per million reads (FPKMs) (Human Body Map 2.0 data).

Table 3 (part II) | Functional characteristics of the identified miRNA binding site-variants in the 3'UTR of 13 genes associated with Parkinson's Disease.

	rs3802921	rs1052594	rs62064654	rs1128402	rs8060857	rs748483
GWAS P value	2.7×10 –7	5.0×10 –8	5.0×10 –8	4.9×10 –7	1.2×10 –7	1.8×10 –16
OR	1.1	>1	<1	0.92	0.91	1.22
Gene Host gene	IGSF9B	MAPT	PLEKHM1	SPPL2B	STX1B	TMEM175
Brain Exp	0.3	54	4.7	44	6.4	5
Proxy All SNPs	5	1067	133	4	55	13
Nonsynon	0	12	1	0	1	0
eQTL Blood	–	–	–	–	–	–
Brain	–	–	–	1.1×10 –9	–	–
miR-binding site Dis (A1)	miR-489-3p	NC	miR-339-3p	None	miR-6867	None
ΔScore	0.02	–	–0.19	–	0.14	–
Cre (A2)	miR-514-3p	miR-486-3p	miR-657	miR-29a-5p	None	miR-5003
ΔScore	0.1	–0.03	–0.09	0.03	–	–0.15

Supporting evidence for 13 miRNA binding site-variants that are associated with PD. For genes hosting more than one variant in miRNA binding sites, a variant with a higher probability of being functional is reported in the table. In addition, for variants that are predicted to affect more than one miRNA, the highly conserved miRNA in each situation is mentioned (full information provided in the Supplementary data). miR, miRNA; OR, Odds ratio; Exp, Expression; Nonsynon, Non-Synonymous SNPs (using the HaploReg web tool v3); ΔScore, Context score change (using the PolymiRTS database v3); Dis, Disruption binding site; Cre, Creation binding site; A1, Allele 1; A2, Allele 2; None, No binding site for miRNAs; NC, Binding site for a non-conserved miRNA; NA, Not available. The value for eQTL is p-value; the value for brain expression is fragments per kb of exon per million reads (FPKMs) (Human Body Map 2.0 data).

DISCUSSION

In this study, we investigated the association of variants that are located in miRNAs and miRNA binding sites with PD using population-level data. We found two common miRNA variants, rs897984 in miR-4519 and rs11651671 in miR-548at-5p, that are associated with PD. We showed that the variant's mutant alleles have the potential to affect the hairpin secondary structures of pre-miRNAs and decrease the expression levels of mature miR-4519 and miR-548at-5p. We subsequently suggested target genes that might mediate the effects of miR-4519 and miR-548at-5p on PD. Among them, we experimentally showed that *NSF* is a direct target of miR-4519. Furthermore, we identified 32 miRNA binding site-variants that are associated with PD and could potentially affect miRNA-mediated regulation of their host genes. Four of the host genes, *CTSB*, *STX1B*, *IGSF9B* and *HSD3B7*, had not previously been reported to be associated with PD. Finally, we provide supporting evidence for some of the identified miRNA binding site-variants to be functional in their loci.

An increasing number of studies have shown the critical role of miRNAs in neuro-degenerative disorders including PD.[35-37] Most of these studies have mainly focused on differentially expressed miRNAs and genes detected by expression arrays in a small sample size. In addition, some studies have linked a number of miRNAs with neurodegenerative disorders using the candidate gene approach. For example, Saba et al. have recently reported a catalog of SNPs overlapping miRNA-binding sites in a subset of genes that are implicated in neurological diseases[38] Here, we systematically investigated the association of all miRNA-related genetic variants with PD using population level data in a large scale.

The GWAS data shows that carriers of the rs897984 mutant allele in miR-4519 have a decreased risk of PD. Our functional experiments then showed that the mutant allele dramatically decreases the expression level of mature miR-4519. rs897984 occurs 1 nt after the 5′ end of pre-miR-4519 and overlaps the site of Drosha cleavage. The maturation of miRNAs is a complex and highly regulated process, which is characterized by two-step sequential processing by RNase III enzymes, Drosha and Dicer.[39,40] It has been shown that polymorphisms residing within (±)1 nt of the Drosha or Dicer cleavage sites may affect miRNA biogenesis.[41,42] Therefore, the observed effect of rs897984 on the miR-4519 level can be explained with the SNP impact on the processing of miR-4519 by Drosha enzyme. We showed that miR-4519 is expressed in both gray and white matter of the human brain. Interestingly, data from the GTEx database (http://www.gtexportal.org/home/gene/mir4519) showed that miR-4519 is expressed in higher levels in substantia nigra, the primary area of the brain that is affected by PD [43] In agreement with our findings, two recent studies have also demonstrated this miRNA to be expressed in the human brain tissues.[44,45]

We suggested that the effect of miR-4519 on PD might be mediated by four target genes (*NSF*, *TMEM163*, *CCNT2* and *SH3GL2*) that were found to be potentially associated with PD. Among them, *NSF* showed the most significant association with PD (in the GWAS data) and is the most abundantly expressed gene in the brain (in the Human Body Map data). We further experimentally showed that *NSF* is a direct target of miR-4519. *NSF* is known as a crucial factor in intracellular membrane-fusion events, such as the fusion of synaptic vesicles with the presynaptic membrane during neurotransmission.[46] This gene has also been shown to be associated with different neurodegenerative disorders including PD.[47,48] Furthermore, previous studies have shown that expression of *NSF* is

decreased in PD substantia nigra.[49-51]These data may indicate that the rs897984 mutant allele influences the risk of PD through reducing the level of miR-4519 and increasing the expression of *NSF* gene.

We found that the rs11651671 mutant allele in miR-548at-5p is associated with an increased risk of PD. *In silico* analysis indicated that the SNP rs11651671 may affect the hairpin structure of precursor miR-548at-5p. In agreement with this conjecture, our functional experiments demonstrated that the mutant allele significantly reduces the level of miR-548at-5p. Our qPCR data did not show the expression of miR-548at-5p in the human brain samples. However, *ATP6V0A1*, which hosts miR-548at-5p in a sense direction, is abundantly expressed in the brain (Human Body Map data). Previous studies have suggested that the expression of an miRNA host gene can be used as a proxy for miRNA expression when both the host gene and the miRNA are in the same direction.[52] Future studies are thereby needed to examine the miR-548at-5p expression in the more relevant tissues (such as substantia nigra) in the brain. Moreover, we found four miR-548at-5p target genes, *GCH1*, *MMRN1*, *CCNT2* and *DCUN1D1*, that are associated with PD and are likely to be potential mediators of the miRNA effects on PD. Among them, *GCH1*, also called Dopa-Responsive Dystonia1, has been shown to be associated with movement disorders including early-onset PD.[53-55] These results may indicate that a decreased level of miR-548at-5p in carriers of the rs11651671 mutant allele influence the risk of PD through altering the expression of these target genes.

We identified 32 miRNA binding site-SNPs that are associated with PD. Among 13 genes hosting the variants, *SNCA* (α-synuclein) plays an important role for normal brain function and is a major risk factor for PD.[56] Genetic variants in *SNCA* have been shown to be associated with the common sporadic form of PD.[57-59]

However, some of the associated variants are mapped downstream of the *SNCA* gene and a direct functional effect is thus unlikely. rs356165 is located in the 3'UTR of *SNCA* and is one of the top associated SNPs with PD in the related locus. We found that the rs356165 mutant allele is predicted to disrupt a binding site of miR-6508, presumably resulting in an elevated level of *SNCA* expression. An allele-specific regulation of *SNCA* by miR-6508 might serve as a functional explanation behind the association of rs356165 with PD. Future studies are needed to determine the effect of rs356165 on *SNCA* expression and the function of miR-6508 in PD patients. Conversely, our results showed that some of the identified miRNA-binding site SNPs improve the original recognition sites or create new binding sites for miRNAs. For example, rs1128402 is predicted to create a binding site for miR-29a-5p in the 3'UTR of *SPPL2B*. This is one of the top SNPs associated with PD in the related locus and has no non-synonymous proxy. In addition, several independent studies have shown that miR-29a-5p is involved in PD and other neurodegenerative diseases.[60-63] Down-regulation of *SPPL2B* by miR-29a-5p may be a functional reason for the identified association of rs1128402 with PD.

We suggested four new genes, *CTSB*, *STX1B*, *IGSF9B* and *HSD3B7*, that are potentially associated with PD. Of these, *CTSB* (Cathepsin B1) encodes a protein that is known as amyloid precursor protein secretase and is involved in the proteolytic processing of amyloid precursor protein (APP). Incomplete proteolytic processing of APP has been suggested to be a causative factor in Alzheimer disease.[64,65] We found three miRNA-binding site SNPs in the 3'UTR of *CTSB* that are associated with PD. Of these, rs2645425 is predicted to create a potential binding site for miR-342-3p. Our blood eQTL analysis showed that rs2645425 mutant allele carriers have lower blood expression levels of *CTSB*. The expression

data further demonstrated that the *CTSB* gene is abundantly expressed in the brain. Interestingly, miR-342-3p has also been shown to be upregulated in the brain of patients with prion disease[62,66] and has been suggested as a biomarker for Alzheimer's Disease.[67] The observed association of rs2645425 with PD could be through increasing the miR-342-3p-dependent regulation of *CTSB*. Among the other three genes, *STX1B* (syntaxin-1B) has been found to be directly implicated in the process of calcium-dependent synaptic transmission in the rat brain and has been suggested to play an important role in the excitatory pathway of synaptic transmission.[68] *IGSF9B* is known as Immunoglobulin Superfamily Member 9B and has reported as a novel, brain-specific, homophilic adhesion molecule that is strongly expressed in GABAergic interneurons.[69] Finally, *HSD3B7* has been shown to be associated with congenital bile acid synthesis defect and liver diseases.[70] This gene has also been linked with the etiology of Alzheimer disease through deactivation pathway of LXR ligands in the brain.[71]

Collectively, we identified two miRNA variants and multiple miRNA binding site variants that are associated with PD and could affect miRNA-mediated regulation of several genes associated with PD. These results may contribute to increase our understanding of the role of miRNAs in the etiology of PD. Our findings may also be of clinical importance as they suggest a number of miRNAs that modify gene expression profiles and affect PD risk. Experimental assays of the identified variants and miRNA profiling in PD patients will be the next step towards determining the functionality of the variants, related target genes, and miRNAs in PD.

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Chapter 6

Parkinson's Disease: non-genetic predisposition

Three other workmen employed in grinding manganese have been similarly affected; but in them the disease was arrested by removing the cause.

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Chapter 6.1

Non-motor risk factors

ABSTRACT

At present, there are no validated methods to identify individuals who are at increased risk for Parkinson's Disease (PD) from the general population. We investigated the clinical usefulness of a recently proposed non-motor risk score for PD (the PREDICT-PD risk score) in the population-based Rotterdam Study. At baseline (1990), we constructed a weighted risk score based on 10 early non-motor features and risk factors in 6,492 individuals free of parkinsonism and dementia. We followed these individuals for up to twenty years (median 16.1 years) for the onset of PD until 2011. We studied the association between the PREDICT-PD risk score and incident PD using competing risk regression models with adjustment for age and sex. In addition, we assessed whether the PREDICT-PD risk score improved discrimination (C-statistics) and risk classification (net reclassification improvement) of incident PD beyond age and sex. During follow-up, 110 individuals were diagnosed with incident PD. The PREDICT-PD risk score was associated with incident PD (hazard ratio [HR]=1.30; 95% confidence interval [1.06; 1.59]) and yielded a small, non-significant improvement in overall discrimination (ΔC-statistic=0.018[-0.005;0.041]) and risk classification (net reclassification improvement=0.172[-0.017;0.360]) of incident PD. In conclusion, the PREDICT-PD risk score only slightly improves long-term prediction of PD in the community.

BACKGROUND

Parkinson's Disease (PD) is the second most common neurodegenerative disorder among elderly.[1] At present, no treatment can effectively modify disease progression in patients with PD. This may be due to the advanced stage of pathology that PD patients already have at the time of clinical diagnosis.[2] The identification of individuals from the general population who are at high risk of PD might open the door to earlier diagnosis, and possibly enable early symptomatic treatment. Equally important, it would enable the selection of individuals who, possibly after additional refined screening, can be enrolled in neuroprotective trials.

In the most recent comprehensive meta-analysis of non-motor features and risk factors for PD to date, several variables were determined to affect the risk of PD.[3] Subsequently, a cohort study in the United Kingdom was initiated (PREDICT-PD) to assess the validity of a risk score based on 11 of these variables to prospectively predict PD. In cross-sectional analyses at baseline, the PREDICT-PD risk score was associated with several proxies for PD.[4] However, the prospective usefulness of the PREDICT-PD risk score for PD remains unclear.

Here, we investigated the prospective prognostic value of the PREDICT-PD risk score in an independent, population-based sample with twenty years of follow-up.

METHODS

Study design and setting

The study was embedded in the first subcohort of the Rotterdam Study (RS-I), a large, prospective, population-based study in the Netherlands.[5,6] The study was initiated in 1990, inviting all inhabitants of Ommoord who were aged ≥55 years. 7,983 participants (78%) agreed to participate and provided written informed consent. At baseline, participants were extensively screened for parkinsonism and dementia, and assessments of non-motor features and risk factors used to derive the risk score were conducted.[7,8] For this report, we excluded individuals with prevalent parkinsonism or dementia and individuals who were not screened for both, leaving 6,492 individuals for analyses. We followed participants for the development of PD from baseline until: onset of parkinsonism, onset of dementia, death or 1 January 2011, whichever came first. Until 2011, the study has had a total of five visits, including four follow-up visits. At each visit, participants underwent home interviews and medical examinations at the research center.

Assessment of parkinsonism and PD

A detailed description of assessment methods is provided in **Chapter 4.1**. In short, we used four overlapping modalities to screen for potential parkinsonism: in-person examinations (on average every 4 years), in-person interviews, use of antiparkinson medication, and clinical monitoring alerts.

Of all individuals who screened positive in any of these methods, complete medical records (including letters from medical records of specialists and general practitioners) were studied and case reports were drawn up covering all potentially relevant information to establish presence and cause of parkinsonism. These case reports were evaluated by a panel led by an experienced neurologist.

Parkinsonism was defined by presence of hypo- or bradykinesia in combination with ≥1 cardinal sign (resting tremor, rigidity or postural imbalance) observed by any physician, or a clinical diagnosis of parkinsonism by a neurologist or geriatrician (if motor examination details were unavailable). PD was only diagnosed after exclusion of secondary causes, in individuals with at least one of: 1) a clinical PD diagnosis by a neurologist or geriatrician; 2) positive response to dopaminergic treatment. Individuals with parkinsonism who did not fulfill PD criteria were considered secondary parkinsonism cases. After initial diagnosis, medical records of all incident parkinsonism cases continued to be scrutinized until the end of the study period for new information that could lead to a revision of the diagnosis.

Assessment of non-motor features and risk factors in the PREDICT-PD risk score

Non-motor features and risk factors used to derive the risk score were assessed during the baseline home interview and center visits. Smoking habits were assessed during home interviews and participants were subsequently categorized as current, former and never smokers. Coffee and alcohol intake were assessed using food-frequency questionnaires. In addition, participants were asked whether any of their parents, siblings or children had PD. Participants were also asked: "Did you ever have a serious head trauma or a concussion?" and "Did you ever have periods of depression?".

During home interviews, participants were questioned for current medication they were using at the time. This included laxative medication, non-steroidal anti-inflammatory drugs (NSAIDs), calcium-channel blockers, beta-blockers, and other

antihypertensive drugs (ATC-codes C02, C03, C07, C08, and C09). Since we had no data available on stool frequency, we considered use of laxative medication as a proxy for constipation. Blood pressure was measured twice during center visits, and hypertension was diagnosed if the mean of two measurements exceeded 140/90mmHg or if an individual used antihypertensive medication with an adequate indication.

We had no data on erectile dysfunction and consequently excluded erectile dysfunction from the risk score. In the meta-analysis, farming occupation, rural living, pesticide exposure, and well-water drinking were also identified as risk factors,[3] but these factors were not included in the PREDICT-PD risk score.[4] In the Rotterdam Study, only 5 study participants (<0.1%) worked as a farmer (none of whom developed PD during follow-up), and all study participants lived in a non-rural, suburban district (i.e., Ommoord). We lacked information on pesticide exposure and well-water drinking.

Statistical analysis

We constructed a risk score for each individual, by adding up their number of risk factors weighted by the log-transformed, reported risk-increasing or (inverted) risk-decreasing effect size for the association with PD.[3] Risk scores were transformed into z-scores to facilitate evaluation of their effect per standard deviation increase. A higher risk score corresponds to a larger weighted number of risk factors and thus a higher expected risk of PD. We constructed two models: model I comprised age and sex for overall analyses, and only age for sex-stratified analyses. Model II comprised model I plus the PREDICT-PD risk score. We visually

inspected reclassification of risk after addition of the PREDICT-PD risk score using a reclassification scatterplot.[9]

We investigated the association between the risk score and incident PD by comparing model II to model I using the method proposed by Fine and Gray, which takes into account the risk of competitive events (i.e., incident dementia or death).[10] To assess effect modification by sex, we examined the interaction term of the PREDICT-PD risk score with sex, and subsequently stratified analyses by sex. The discriminative value of both models was expressed with Uno's C-statistic, which takes into account right-censoring.[11] To study reclassification, we calculated the continuous net reclassification improvement (NRI).[12] Since the predictive power of dependent-state risk factors may decrease over time, we repeated our prediction analyses after restriction of follow-up to the first 5 and 10 years, respectively.

In post-hoc analyses, we examined effect modification of the PREDICT-PD risk score by genetic predisposition for PD, using a genetic risk score that we previously reported on,[13] by adding the genetic risk score and an interaction term of [PREDICT-PD risk score*genetic risk score] to the main model. Furthermore, we assessed non-linear effect modification of the association between the PREDICT-PD score and incident PD by genetic predisposition by dichotomizing both risk scores at threshold [z-scores > 1]. Furthermore, we explored effect modification by separate genetic variants on the association of environmental and medication risk factors (from the PREDICT-PD score) with incident PD.

We had complete data on 91% of predictor values (missing values between 0-19% per predictor). Missing values were handled by multiple imputation using the mean of five imputations, based on age, sex and all other non-motor predictors.

RESULTS

The most prevalent non-motor risk factors were coffee and alcohol use, while constipation and a family history of PD were the least prevalent.(*Table 1*) During follow-up (87,321 person-years, median 16.1 years), 110 individuals had incident PD (age-adjusted incidence rate 1.4 per 1,000 person-years) of whom 56 were men and 54 were women. In total, 3,713 individuals died, and 1,021 were diagnosed with incident dementia while at risk of parkinsonism. In our population, the only risk factors that were independently associated with incident PD were current smoking, former smoking and depression.(*Table 1*)

As shown in *Online Resource 2*, women had effect estimates of laxative use, family history, hypertension, NSAID use, CCB use, and alcohol for incident PD that were direction-consistent with the meta-analysis, whereas men had opposite estimates. Furthermore, we observed a significant association between family history and incident PD in women, but not in men. Predicted 20-year risk of PD ranged from 0.7% to 18.8% in model I (median 2.2%), and from 0.5% to 22.5% (median 2.2%) in model II.(*Figure 1*) During follow-up, individuals in the highest PREDICT-PD risk score tertile consistently had the highest cumulative hazard of incident PD.(*Online Resource 3*) The PREDICT-PD risk score was independently associated with incident PD and yielded a small, non-significant improvement in discrimination of incident PD beyond age and sex.(*Table 2*; $\Delta C=0.018$ [-0.005; 0.041]) Compared to model I, model II slightly improved overall classification of PD risk. The association between the PREDICT-PD risk score and incident PD was strongly modified by sex ($p=0.004$). Stratified analyses showed that the risk score was associated with incident PD independently of age in women but not in men.

Table 1 | Study population characteristics.

Characteristic	N in the Rotterdam Study	Reported RR/OR*	HR (95% CI) in Rotterdam Study
Age at baseline, mean, y (SD)	68.7 (8.7)	-	1.03 (1.01; 1.05)
Women (%)	3818 (58.8)	-	0.39 (0.24; 0.62)
Smoking (%)			
Never	2202 (34.6)	1.00	
Former	2695 (42.4)	0.78	0.53 (0.32; 0.89)
Current	1463 (23.0)	0.44	0.36 (0.19; 0.67)
Family history (%)**	311 (5.0)	4.45	1.62 (0.80; 3.27)
Coffee (%)	5087 (97.2)	0.67	1.78 (0.38; 8.27)
Alcohol (%)	4154 (79.4)	0.90	0.87 (0.52; 1.44)
Hypertension (%)	3572 (55.0)	0.74	1.13 (0.74; 1.73)
NSAID use (%)	512 (7.9)	0.83	1.14 (0.58; 2.24)
CCB use (%)	388 (6.0)	0.90	1.42 (0.75; 2.69)
Beta-blocker use (%)	948 (14.6)	1.28	1.20 (0.72; 2.00)
Constipation (%)	237 (3.7)	2.34	1.35 (0.58; 3.13)
Head injury (%)	1980 (30.5)	1.58	0.77 (0.51; 1.18)
Depression*** (%)	2028 (33.2)	1.86	1.63 (1.10; 2.42)

*N, number of individuals at risk of Parkinson's Disease. RR, relative risk. OR, odds ratio. HR, hazard ratio adjusted for age, sex and all other risk factors. 95% CI, 95% confidence interval. y, year; SD, standard deviation. NSAID, non-steroidal anti-inflammatory drug. CCB, calcium channel blocker. For constipation, a proxy was used (use of laxative medication). *Reported in the meta-analysis of early non-motor features and risk factors by Noyce et al 1. Of note, no relative risks or odds ratios were reported for age and sex. **History of Parkinson's Disease in parents, siblings or children.* ***Self-reported periods of depression.

In line with this, risk prediction of PD based solely on age was more accurate in men than in women, but this difference faded after application of the PREDICT-PD risk score. Classification of PD risk was improved by model II in women, but not in men.

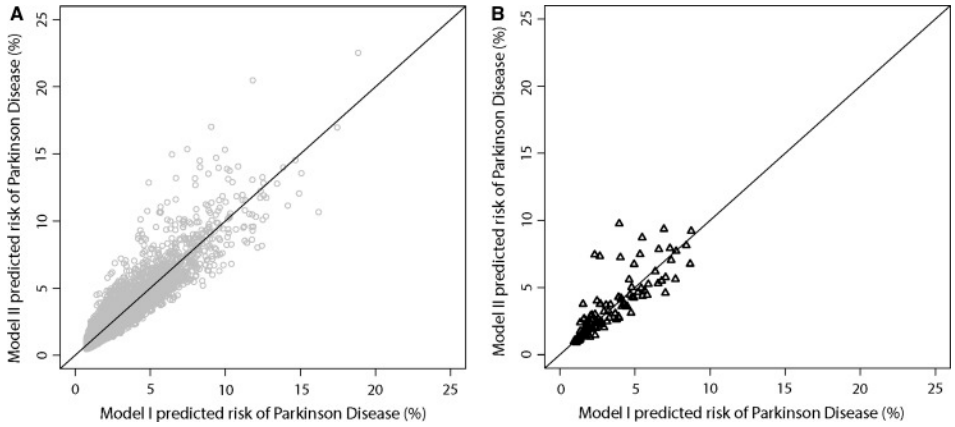


Figure 1 | Reclassification scatterplot of the 20-year risk of incident Parkinson's Disease after addition of the PREDICT-PD risk score.
Model I, overall: age and sex. Model II, overall: age, sex and PREDICT-PD risk score. Fig 1A. Individuals without incident Parkinson's Disease. Fig 1B. Individuals with incident Parkinson's Disease.

After restriction of follow-up to 5 years, discrimination and risk classification of incident PD did not significantly improve (ΔC=0.008 [-0.022; 0.037] and risk classification (NRI=0.012 [-0.091; 0.145]) from model I to II. Similarly, after restriction of follow-up to 10 years, prediction did not improve (ΔC=0.013 [-0.011; 0.038] and NRI=0.031 [-0.069; 0.140]).

The combination of the genetic risk score and PREDICT-PD risk score modestly improved prediction of PD beyond age and sex (ΔC-statistic=0.03 [0.00; 0.06]). We observed no statistically significant evidence for linear effect modification between both risk scores. However, at threshold [z-scores > 1], elevated genetic risk and elevated non-motor risk had effect-modifying effects on the risk of PD (p for interaction term=0.04). Of note, these findings were not statistically significant after taking into account multiple testing.

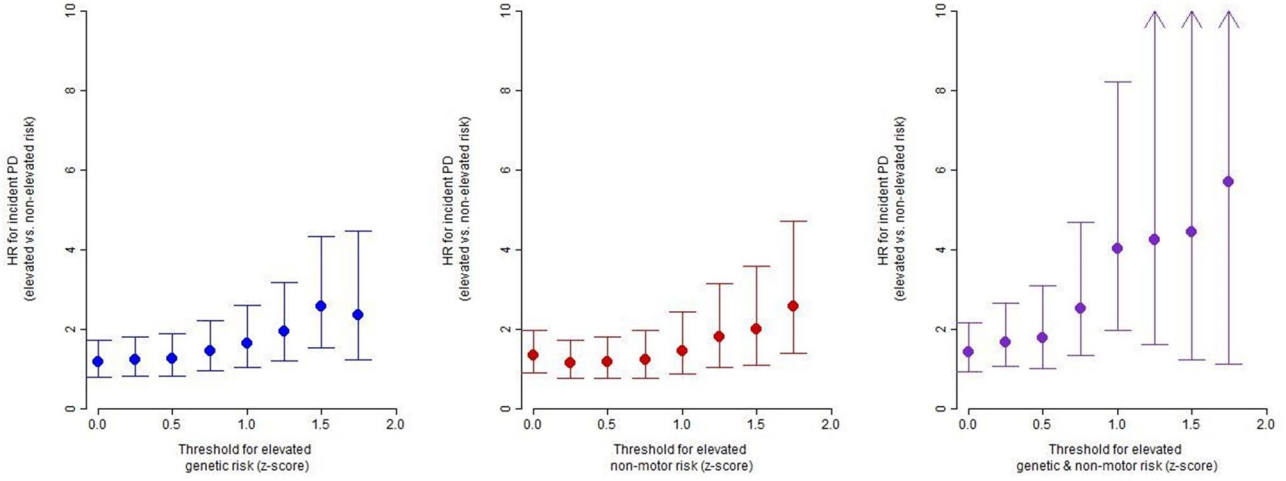
Table 2 | PREDICT-PD risk score and 20-year risk of incident Parkinson's Disease.

Group	Model	Association	Discrimination	Reclassification
		HR	*C-statistic*	*NRI*
		[95% CI]	*[95% CI]*	*[95% CI]*
Overall	I	(Reference)	0.649 [0.592; 0.707]	(Reference)
	II	1.30 [1.06; 1.59]	0.667 [0.609; 0.725]	0.172 [-0.017; 0.360]
Men	I	(Reference)	0.684 [0.617; 0.752]	(Reference)
	II	0.90 [0.63; 1.30]	0.681 [0.605; 0.758]	-0.105 [-0.356; 0.145]
Women	I	(Reference)	0.604 [0.530; 0.677]	(Reference)
	II	1.70 [1.36; 2.12]	0.674 [0.602; 0.746]	0.461 [0.202; 0.721]

Model I, overall: age and sex. Model I, stratified analyses by sex: age. Model II, overall: age, sex and PREDICT-PD risk score. Model II, stratified analyses by sex: age and PREDICT-PD risk score. HR, hazard ratio for incident Parkinson's Disease per standard deviation in risk score. CI, confidence interval. NRI, continuous net reclassification improvement (model I is reference).

In *Figure 2*, the age-&sex-adjusted hazard ratios for incident PD are shown across increasing (z-score) thresholds of elevated genetic risk, elevated non-motor risk, and both. In separate analyses of effect modification of environmental and medication risk factors (which are part of the non-motor risk score) by genetic predisposition, we identified suggestive effect-modifying effects on the risk of PD by *MIR4697* of the association of NSAID use (p=0.04) ; by *GBA* (p=0.03) and by *GCH1* (p=0.03) of the association of coffee use with incident PD; and by *SNCA* of the association of both NSAID (p=0.04) and beta-blocker (p=0.03) use with incident PD.

Figure 2 | Genetic and non-motor nongenetic factors on the risk of incident Parkinson's Disease.



Analyses were adjusted for age and sex. HR, hazard ratio. PD, Parkinson's Disease. Non-motor risk score, PREDICT-PD.

DISCUSSION

In this prospective, population-based sample with twenty years of follow-up, we found that the PREDICT-PD risk score yielded a small, non-significant improvement in overall discrimination and classification of incident PD. This was due to improvement of PD risk prediction in women to the level of men.

At present, there are no validated methods to identify individuals at high risk for PD from the general population so that they can be monitored for onset of symptoms or enrolled in neuroprotective trials. The recently proposed PREDICT-PD risk score was based on a meta-analysis of early non-motor features and risk factors. [3,4] Strengths of our study were its prospective design and inclusion of community-dwelling individuals irrespective of PD risk. Compared to cross-sectional case-control data, such as from the multi-center Parkinson's Progression Marker Initiative,[14] prospective population-based studies such as the Rotterdam Study have the advantage that all participants (i.e., both PD future cases and controls) were included and followed up using the same methodology, presumably ensuring a realistic estimate of the risk of incident PD in the general population. Further strengths include long duration of follow-up for PD (median 16.1 years) and standardized assessment of PD diagnosis. In addition, our sample was completely independent of discovery samples used for relative risk estimates in the meta-analysis.[3]

Limitations included lack of data on erectile dysfunction as well as the assessment of head trauma and depression using a single question. In addition, we used laxative medication as a proxy for constipation, which likely caused a severe underestimate of the true prevalence of constipation, since many people who suffer from constipation do not use drugs and change their dietary and lifestyle

habits. In our sample, only a small proportion of male participants who did not develop incident PD and not a single male incident PD patient used laxatives at baseline, suggesting that our underestimate may have been larger in men than in women. If we would have had complete information on these factors, the PREDICT-PD risk score may have improved PD prediction significantly in our population. Furthermore, we lacked histologic confirmation of specific pathologies, which may have introduced some non-differential misclassification of PD cases. Also, we may have been underpowered to detect a small significant improvement in PD prediction, especially in the middle-long term (i.e., 5 years). The estimates used in the PREDICT-PD risk score were based on studies that did not assess all variables simultaneously, and the estimates were not sex-specific. In our sample, only 3 risk factors were independently associated with incident PD (current smoking, former smoking and depression), which may indicate that the meta-analyzed estimates were inflated due to limitations of the meta-analysis, such as publication bias, a substantial degree of selection in some discovery samples, or insufficient adjustment for covariates.[3] Alternatively, we may have been underpowered to detect significant associations with PD for separate risk factors, and limitations in our assessment methods may have led to underestimates of true associations. Future collaboration across cohort studies who have prospectively assessed (nearly) all risk variables in the score will probably increase the accuracy of risk estimates. Similarly, while we observed clear sex differences in associations between risk factors and incident PD, most of the sex-specific associations in our sample were non-significant. Collaborative studies may distinguish true sex differences from limitations in assessment methods that may have worse in men (e.g., laxative use).

Future studies can further build on the PREDICT-PD risk score by focusing on three other key aspects. First, some relatively common non-motor risk factors for PD were not yet part of the risk score, such as impaired olfactory function.[15] Recently, dedicated olfactory function testing was shown to distinguish patients with a PD diagnosis from controls with very high accuracy.[14] Although the long-term prospective predictive value of olfactory testing for PD in the community has not yet been demonstrated empirically, a previous study showed that impaired olfaction is associated with PD up to 4 years before clinical diagnosis.[15] Therefore, inclusion of prospective measures of olfactory function in the risk score may further improve prediction of PD in the community. Second, while the Rotterdam Study comprises a suburban-based study population with only few farmers, discrimination and classification accuracy in other communities may be improved by inclusion of data on rural living and farming occupation. Third, motor features were not included in the risk score. Even in the absence of objective signs on routine screening, prediagnostic PD patients have subjective parkinsonian complaints more frequently than controls,[16] and tremor is the most common presentation of PD patients in primary care practice ten years before clinical diagnosis.[17] The advancement of dedicated motor screening tests might not only lead to reliably detection of PD in select subgroups of very high-risk individuals (e.g., RBD-patients[18]), but potentially also in community-dwelling individuals. Furthermore, post-hoc analyses showed that adding genetic data yields a small improvement in the accuracy of PD risk prediction, although predictive measures were still moderate at best. We also note that the PREDICT-PD score not only included putative causal factors, but also prodromal features.

In conclusion, the PREDICT-PD risk score is a small step forward towards predicting incident PD in the community, in particular in women, but there is still a clear need for improvement.

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Chapter 6.2

Vascular pathology

Chapter 6.2

ABSTRACT

Parkinsonism is a common neurodegenerative syndrome in middle-aged and elderly individuals. The etiology is multifactorial and may include a vascular contribution. We aimed to determine whether markers of subclinical vascular pathology are associated with the risk of all-cause parkinsonism in the general population. We assessed a range of markers of subclinical vascular pathology (ankle brachial index, carotid plaques and intima media thickness, retinal arteriolar and venular calibers) in 6,199 individuals from the population-based Rotterdam Study, who were free of parkinsonism and dementia at baseline. We followed these individuals up till onset of parkinsonism, dementia, and death for 89,387 person-years until January 1, 2013. Hazard ratios (HRs) for all-cause parkinsonism and separately for Parkinson's Disease (PD) versus non-PD were estimated from competing risk regression models adjusting for potential confounders. During follow-up, we identified 211 cases of parkinsonism (110 had PD). None of the five markers of subclinical pathology was associated with all-cause parkinsonism. Only low ankle brachial index was associated with a higher risk of non-PD parkinsonism (HR=0.79, 95% CI: 0.68-0.92), but not with the risk of PD. We did not find a consistent pattern of associations between systemic vascular pathology markers with parkinsonism, suggesting that the potential involvement of vascular pathology is not prominent or needs further evaluation in studies with an even larger sample size.

BACKGROUND

Parkinsonism, with its most frequent subtype Parkinson's Disease (PD), is a common chronic neurodegenerative syndrome in the elderly.[1,2] Although many genetic and environmental factors have been implicated in the multi-factorial etiology of parkinsonism,[1] there is still much uncertainty about the exact mechanisms underlying neuronal cell loss in patients with PD and non-PD parkinsonism. There is a scarcity of knowledge on early markers and potential neuroprotective therapies in early stages of parkinsonism, and patients are typically already in an advanced pathological disease stage when the diagnosis is made.[1] Therefore, there is a need for novel insight into factors associated with all-cause parkinsonism.

Markers of subclinical vascular pathology are strongly implicated in the etiology of the two common neurological syndromes, stroke and dementia.[3] Furthermore, a high prevalence of lacunar infarcts have been reported in the cerebral white matter and basal ganglia of patients with parkinsonism,[4] which indicates a potential vascular etiology. During the course of Alzheimer's Disease 25% of patients develop parkinsonism,[5] whereas approximately 30% of patients with PD are eventually diagnosed with dementia.[6] However, in spite of an overlap in clinical and pathological findings between primary parkinsonism (such as PD) and dementia or stroke, the role of vascular pathology in the etiology of parkinsonism syndromes remains unclear.

Vascular pathology can be non-invasively measured both in large systemic vessels as well as in microvasculature locally.[7] Commonly used, non-invasive measurements of vascular pathology of the large vessels include ultrasound measures of carotid arteries (intima media thickness and plaques) and ankle

brachial index (ABI).[8,9] Furthermore, structural changes in the retinal vasculature have long been recognized as an important marker of microvascular pathology.[10] These non-invasive measures provide an insight into the condition of the large and small blood vessels.

We hypothesized that subclinical vascular pathology is associated with an increased risk of parkinsonism in the general population. We used noninvasive measurements of subclinical vascular disease to test our hypothesis in a large, population-based cohort study.

METHODS

Setting and study population

This study was conducted as part of the Rotterdam Study, a population-based prospective cohort study in elderly adults.[11] There were only two selection criteria: individuals had to live in the well-defined Ommoord district of Rotterdam in 1990, and they had to be aged 55 years or older. Out of 10,215 eligible individuals, 7,983 agreed to participate and provided informed consent.

All participants were interviewed and underwent extensive physical examination at the research center, including screening for parkinsonian signs and markers of vascular pathology at baseline (1990-1993) and at four follow-up rounds (1993-1995, 1997-1999, 2002-2004, and 2009-2011).

For the current study we excluded participants with prevalent parkinsonism (n=126), incomplete neurological screening (n=1401) at baseline, and participants with missing information regarding two or more vascular pathology markers

(n=257), leaving 6,199 individuals at risk to develop any kind of parkinsonism during the follow-up period.

The overall age- and sex-adjusted incidence rate of PD is 1.5 per 1,000 person-years in the Rotterdam Study, which is similar to other population-based studies, while age-specific incidence rates are somewhat higher in the Rotterdam Study.[12]

Assessment of subclinical vascular disease

Recently, CT and MRI assessments were implemented in the Rotterdam Study; however, we lacked sufficient follow-up for incident parkinsonism to incorporate these measurements in our present analyses. Subclinical vascular pathology was assessed with four different measures: ankle-brachial index, carotid intima-media thickness and the presence of plaques in the carotid arteries assessed using ultrasonography and retinal vessels diameters.

Ankle-brachial index: We assessed ankle-brachial index (ABI) by measuring systolic blood pressure (SBP) in all extremities. The SBP was measured twice in both arms and at both ankles (posterior tibial artery) in each participant in the supine position with an 8-MHx continuous-wave Doppler device (Huntleigh D500, Hunteleigh Technology) which was attached to a standard random-zero sphygmomanometer.[8] To obtain the ABI we calculated the ratio of the systolic blood pressure at the ankle and the systolic blood pressure at the arm per side. ABI values higher than 1.50 were not included in the study, because that values are considered irrationally high due to calcification of the blood vessels and their inability to properly compress. We used the lowest value of the two sides for the analyses.[8]

Carotid number of plaques and intima media thickness: Using B-mode ultrasonography we evaluated both carotid arteries for the presence of plaques and determined the carotid intima media thickness (cIMT).[9] The presence of a plaque was defined as a focal widening relative to bordering segments, with protrusion into the lumen consisting of either echolucent or echogenic areas. We assessed the presence of plaques at both sides in the common carotid artery, carotid bifurcation and proximal part of the internal carotid artery. The total amount of plaques was reflected in a weighted plaque score that ranged from 0 to 6.[13] The average of the maximum IMT was registered at both the near- and far wall of the common carotid artery for assessing the cIMT.

Retinal vessel measurements: At the baseline ophthalmic examination, simultaneous stereoscopic fundus color transparencies were taken centered on the optic disk (20° field, Topcon Optical Company, Tokyo, Japan) after pharmacologic mydriasis and were digitized with a high-resolution scanner (Nikon LS-4000, Nikon Corporation, Japan).[14] Evaluated measures for arteriolar and venular calibers were based on improved Parr-Hubbard formulas and were corrected for magnification changes due to refractive errors of the eye.[14]

Since each eye has a different magnification in case of refractive changes, summary vessel measures were additionally adjusted for the refraction and corneal curvature with Littmann's formula to estimate absolute measures.[14]

Other measurements

We assessed cardiovascular risk factors by interview, physical examination and blood sampling.[11] Blood pressure was calculated as the mean of two consecutive

measurements with a random-zero sphygmomanometer at the right brachial artery while the patient was in a sitting position. Serum total cholesterol was determined by an automated enzymatic procedure in a non-fasting blood sample. Serum high density lipoprotein (HDL) cholesterol was measured after precipitation of the non-HDL fraction with phosphotungstate-magnesium. Height and weight were measured and body mass index (kg/m2) was calculated. Non fasting serum glucose levels were measured using the glucose hexokinase method. Smoking habits of participants were categorized as "ever" or "never". Uric acid was determined with a Kone Diagnostica reagent kit and a Kone auto-analyzer from blood samples of participants.

Ascertainment of parkinsonism and Parkinson's Disease

A detailed description of assessment methods is provided in **Chapter 4.1**. In short, we used four overlapping modalities to screen for potential parkinsonism: in-person examinations (on average every 4 years), in-person interviews, use of antiparkinson medication, and clinical monitoring alerts.

Of all individuals who screened positive in any of these methods, complete medical records (including letters from medical records of specialists and general practitioners) were studied and case reports were drawn up covering all potentially relevant information to establish presence and cause of parkinsonism. These case reports were evaluated by a panel led by an experienced neurologist.

Parkinsonism was defined by presence of hypo- or bradykinesia in combination with ≥1 cardinal sign (resting tremor, rigidity or postural imbalance) observed by any physician, or a clinical diagnosis of parkinsonism by a neurologist or

geriatrician (if motor examination details were unavailable). PD was only diagnosed after exclusion of secondary causes, in individuals with at least one of: 1) a clinical PD diagnosis by a neurologist or geriatrician; 2) positive response to dopaminergic treatment. Individuals with parkinsonism who did not fulfill PD criteria were considered secondary parkinsonism cases. After initial diagnosis, medical records of all incident parkinsonism cases continued to be scrutinized until the end of the study period for new information that could lead to a revision of the diagnosis.

Person-time at risk of incident parkinsonism ended at onset of parkinsonism, death, or January 1, 2013. Person-time at risk of incident PD ended at diagnosis of incident parkinsonism, incident dementia, stroke, death, or January 1, 2013.

Statistical analysis

Baseline characteristics are presented as frequencies with percentages for categorical variables or means (standard deviation [SD]) for continuous variables. Potential differences in baseline characteristics between included and excluded individuals were explored using independent t-tests for continuous variables and chi-square tests for categorical variables (*Supplement 2*). The sample size and power calculations were calculated a priori and presented in *Supplement 3*.

The association of measures of subclinical vascular disease with risk of parkinsonism was investigated using competing risk regression models. We analyzed each risk factor separately. All continuous vascular pathology markers were transformed into z-scores to facilitate direct comparison of effect sizes. We performed similar analyses for the risk of PD and non-PD parkinsonism. For non-

PD parkinsonism, we considered PD and death to be competing events; for PD, we considered non-PD parkinsonism, death, stroke and dementia as competing events. All analyses were adjusted for age and sex (model 1). Additionally, we adjusted our analyses for systolic blood pressure, diastolic blood pressure, body mass index, total cholesterol level, HDL cholesterol level, glucose level, smoking status and serum uric acid (model 2). In all analyses involving retinal vessel diameters we adjusted these for each other (venular diameters for arterial diameters and vice versa). To explore possible effect modification in our analysis we separately added interaction terms of sex and age with markers of subclinical vascular pathology (i.e. sex*ABI; age*ABI) and conducted stratified analyses on sex and on age (using mean age as the threshold). To investigate whether association of ABI and non-PD parkinsonism were driven by drug-induced parkinsonism, we repeated analyses for association after excluding 20 medication-induced cases.

In order to assess subclinical vascular disease measures as prodromal markers instead of risk factors, we repeated main analyses after making cut offs in a follow-up time on two years, five years and ten years (Supplement 4). We conducted sensitivity analysis on estimates of the association between subclinical vascular markers and all-cause parkinsonism after censoring cases of unspecified parkinsonism. Moreover, we stratified PD patients into groups with and without subclinical vascular pathology, and compare them according to the age of onset of the disease, UPDRS-III score, treatment and a mortality rate from the date of onset of parkinsonism until the date of censoring.

We had complete data on 95% of independent variables. Missing values were imputed by multiple imputation (n=5), based on sex, age and all others covariables. IBM SPSS Statistics version 21.0 (IBM Corp, Armonk, NY, USA) and R

version 3.2.5 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analyses.

RESULTS

Baseline characteristics of the study population are presented in *Table 1*. The mean age at baseline of the eligible 6,199 people was 68.5 years and 58.7% of the participants was female. A comparison between included and excluded individuals demonstrated that excluded individuals were older, had a significantly higher body mass index, higher ABI, larger cIMT, and higher serum glucose level (Supplement 2. *Table 1*).

Table 1 | Study population characteristics.

Characteristic	Participants (n = 6199)
Age at baseline, years	68.5 ± 8.5
Women, %	58.7
Smoking, %	64.5
Body mass index, kg/m^2	26.3 ± 3.7
Total cholesterol, mmol/L	6.6 ± 1.2
HDL cholesterol, mmol/L	1.3 ± 0.3
Systolic blood pressure, mm Hg	139.2 ± 22.2
Diastolic blood pressure, mm Hg	73.8 ± 11.4
Serum glucose, mmol/L	6.8 ± 2.5
Serum uric acid, µmol/L	321 ± 80.7
Ankle-brachial index	1.0 ± 0.2
Weighted number of plaques, number	1.5 ± 1.6
Carotid intima media thickness, mm	1.0 ± 0.2
Retinal arteriolar diameter, µm	146.7 ± 14.3
Retinal venular diameter, µm	221.7 ± 20.9

HDL, High-density lipoprotein. Values are mean ± standard deviation for continuous variables, percentage for dichotomous variables.

During a total follow-up of 89,387 person-years, [mean (SD), 14.42 (6.5)], we identified 211 patients with parkinsonism, of whom 110 patients were diagnosed with PD. An overview of diagnoses is provided in *Table 2*.

Table 2 | Overview of incident clinical diagnoses of parkinsonism.

	All-cause parkinsonism patients (n = 211)
Parkinson's Disease	110 (52.1%)
Unspecified parkinsonism	61 (28.9%)
Drug-induced parkinsonism	20 (9.5%)
Vascular parkinsonism	8 (3.8%)
Multiple system atrophy	4 (1.9%)
Lewy body dementia	1 (0.5%)
Secondary to dementia	4 (1.9%)
Secondary to a tumor	2 (0.9%)
Progressive supranuclear palsy	1 (0.5%)

n, number of cases during follow-up.

Table 3 shows the association between markers of subclinical vascular disease and the risk of all-cause parkinsonism, PD and non-PD parkinsonism. Overall, no measure of subclinical vascular disease was associated with the risk of all-cause parkinsonism. Correspondingly, none of the vascular markers in our study were associated with risk of PD. We only found a decreased risk for non-PD parkinsonism for participants with higher ABI [HR: 0.79 (95% CI: 0.68-0.92)]. After excluding drug-induced cases, associations of ABI and the non-PD parkinsonism remained almost unchanged [HR: 0.77 (95% CI: 0.63-0.93)]. Furthermore, carotid plaques, cIMT and diameter of retinal vessels were not associated with non-PD parkinsonism. Additional adjustment for cardiovascular risk factors did not substantially alter any of these results (*Table 3*, Model 2). We found no evidence for effect modification by sex or age (all p-interaction>0.73).

Table 3 | Associations of markers of subclinical vascular pathology with the risk of parkinsonism.

Marker	**Parkinsonism n/N= 211/6199**	**PD n/N= 110/6199**	**non-PD n/N= 101/6199**
	HR (95% CI)	HR (95% CI)	HR (95% CI)
		Model 1	
Ankle brachial index	0.93 (0.82-1.06)	1.14 (0.94-1.39)	**0.79 (0.68-0.92)***
Weighted number of plaques	0.90 (0.78-1.05)	0.84 (0.68-1.05)	0.97 (0.80-1.18)
Carotid intima media thickness	0.91 (0.77-1.08)	0.86 (0.68-1.10)	0.97 (0.77-1.22)
Retinal arteriolar diameter	0.94 (0.78-1.13)	0.98 (0.77-1.25)	0.89 (0.68-1.17)
Retinal venular diameter	0.92 (0.77-1.12)	0.96 (0.76-1.23)	0.89 (0.67-1.19)
		Model 2	
Ankle brachial index	0.88 (0.77-1.01)	1.08 (0.87-1.33)	**0.76 (0.65-0.90)**
Weighted number of plaques	0.95 (0.81-1.10)	0.90 (0.72-1.14)	0.99 (0.82-1.20)
Carotid intima media thickness	0.94 (0.79-1.13)	0.91 (0.69-1.19)	0.98 (0.78-1.24)
Retinal arteriolar diameter	1.01 (0.84-1.20)	1.04 (0.82-1.31)	0.96 (0.73-1.26)
Retinal venular diameter	0.93 (0.77-1.12)	1.01 (0.78-1.27)	0.86 (0.64-1.16)

*n, number of cases; N, number of individuals at risk; PD, Parkinson's Disease; HR, hazard ratio; CI, confidence interval; SD, standard deviation per Per 1-SD increase in marker of subclinical vascular pathology. Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, systolic blood pressure, diastolic blood pressure, smoking, body mass index, total cholesterol, HDL cholesterol, glucose, uric acid. *Significant (p<0.05) data in bold.*

Additional analyses of subclinical vascular pathology markers as predictors of all-cause parkinsonism over restricted follow-up periods are presented in the *Online Supplementary 4, Table 3*. In analyses that were restricted on 2-years of follow up, hazard ratios were slightly higher than in the overall analyses. Results from other

time-restricted analyses did not differ from the overall analyses. The *Online Supplementary Table 4* shows the results of main analyses after censoring 61 cases with unspecified parkinsonism. No differences were found between the main results and the results after censoring unspecified parkinsonism cases. PD patients, who were in the top risk-increasing quartile of at least one subclinical vascular pathology marker, had later onset of a parkinsonism comparing with PD patients without vascular pathology. There was no difference regarding to mortality rate, UPDRS-III score and medication use between this two groups.

Table 4 | Associations of measures of subclinical vascular pathology and the risk of all-cause parkinsonism after censoring unspecified parkinsonism cases.

Marker	**All-cause parkinsonism n/N=150/6199**
	HR (95% CI)
	Model 1
Ankle brachial index	0.90 (0.76-1.06)
Weighted number of plaques	0.89 (0.75-1.06)
Carotid intima media thickness	0.94 (0.77-1.14)
Retinal arteriolar diameter	0.94 (0.77-1.16)
Retinal venular diameter	1.03 (0.84-1.27)
	Model 2
Ankle brachial index	0.86 (0.72-1.02)
Weighted number of plaques	0.92 (0.77-1.10)
Carotid intima media thickness	0.97 (0.80-1.19)
Retinal arteriolar diameter	1.01 (0.81 -1.23)
Retinal venular diameter	1.04 (0.83-1.28)

n, number of cases; N, number of individuals at risk; HR, hazard ratio; CI, confidence interval; SD, standard deviation. Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, systolic blood pressure, diastolic blood pressure, smoking, body mass index, total cholesterol, HDL cholesterol, glucose, uric acid.

Chapter 6.2

DISCUSSION

We did not find an association between markers of subclinical vascular disease and the risk of all-cause parkinsonism or PD. For non-PD parkinsonism, we only observed a significant association with lower ABI, but not with any other marker of subclinical vascular disease.

Several limitations of this study need to be discussed. First, we excluded participants who were not screened for parkinsonism at baseline and individuals with missing data on more than two markers of subclinical vascular pathology. Participants with a missing score were older, on average with lower ABI and with higher cIMT compared to those who were eligible for the study. No significant differences were found in other measured characteristics between the two groups. We cannot exclude that selection bias led to slight underestimations of associations between markers of subclinical vascular pathology and parkinsonism. Second, it seems unlikely that unmeasured confounding could account for the null results, but it cannot be completely excluded in observational studies. The role of potential confounders that were assessed was examined in multivariate models, and these were found to have little impact on effect estimates. Third, we used only extracranial measures of subclinical vascular pathology and not the most sensitive markers of subclinical vascular pathology. In the future, population-based studies with adequate follow-up for incident parkinsonism should focus on measures of intracranial vascular pathology imaging, including novel brain-imaging techniques (e.g. calcification imaging on CT, arterial spin labeling on fMRI). Moreover, the majority of unspecified parkinsonism in our study did not have neuroimaging information. Finally, some misclassification of PD as other causes of parkinsonism might have occurred.

This prospective population-based study has several essential and unique strengths. First, all participants were included and followed up using the same methodology, irrespective of their risk of parkinsonism. Second, the large sample size, long follow-up duration and the carefully collected prospective data on exposure variables as well as on potential confounders minimizes misclassification of baseline vascular pathology profiles of study participants. Furthermore, we actively screened participants for parkinsonism at baseline and only included individuals without parkinsonism. Since all assessments of vascular pathology markers took place at baseline, our findings unequivocally reflect the prediagnostic phase of parkinsonism and PD. Finally, our near-complete follow-up for dementia and death allowed us to account for events that possibly interfere with the observation of the parkinsonism and PD.

Previous population-based study has focused mainly on the association between hypertension and the risk of PD.[15] However, there is a little prospective data on the role of subclinical vascular disease in the etiology of parkinsonism in the general population. Moreover, previous observations were focused on whether vascular pathology contribute to the disease severity in already diagnosed PD patients[16,17] or whether vascular disease co-occurred with PD.[18,19] The main novelty of the current study is that we studied markers of several subclinical vascular pathology as risk factors of incident parkinsonism. Remarkably, none of these measures were associated with all-cause parkinsonism and PD.

Carotid pathology as shown on ultrasound was associated with motor and the cognitive dysfunction in PD patients in previous studies.[16,17] Additionally, carotid plaques and higher cIMT have been associated with gait and balance decline over time.[20] Also, patients with vascular parkinsonism have a higher risk for embolic cerebrovascular events.[16] However, another study showed that patients with PD

had less severe carotid lesions with mostly stable calcified plaques compared to the general population.[19] In this study, we investigated the cIMT and number of plaques as potential risk factors of onset of parkinsonism and PD. We observed that ultrasound measurements of carotid artery pathology were not related to a higher risk of all-cause parkinsonism, PD or non-PD parkinsonism.

A commonly used measurement of vascular pathology in symptomatic and asymptomatic individuals with cardiovascular disease is ABI.[8,21] Ankle brachial index is inversely related to the severity of peripheral arterial disease (PAD), which mean that individuals with lower ABI have more expressed pathology of lower extremity arteries.[8] We found that ABI was not related to a higher risk of PD but instead with the risk of non-PD parkinsonism. There are several possible explanations for the link between peripheral arterial disease (PAD) and non-PD parkinsonism, such as oxidative stress as same pathophysiological mechanism[22,23] and that vascular pathology could lead to neurodegeneration outside of the nigrostriatal area in non-PD parkinsonism patients.[24] Autonomic dysfunction can be one of the first symptoms in non-PD parkinsonism patients.[25] Moreover, the autonomic nervous system (ANS) has blood supply from peripheral vessels and PAD might lead to neuronal cell loss of ANS. Vascular parkinsonism (VP) can occur after a stroke, possibly as a result of atherosclerotic thromboembolism. While the percentage of VP cases among non-PD parkinsonism in our study is low (7.9%), we cannot exclude that we misclassified some VP cases as unspecified parkinsonism cases.

The diameter of retinal vessels is mainly associated with cerebral small vessel disease (CSVD).[10] In our study there was no relationship between arteriolar diameters and larger venular diameters with all-cause parkinsonism. This is contrary to two cross-sectional studies and one study in patients with CSVD,

which revealed that measures of CSVD are associated with the incidence of parkinsonian signs.[26-28] Those studies were using more sensitive measures of CSVD, such as white matter volume, number of lacunes, and cerebral microbleeds. However, their follow-up time was nonexistent or short, and their sample size was smaller than in our study. Similar to our results, one case-control study showed that retinal vessel diameter did not differ between PD patients and healthy controls.[29] Additionally, two recent clinicopathological studies observed no difference between the incidence of small vessels disease pathology and stroke in PD cases compared with the general population.[30,31] Different methodology and different types of parameters for CSVD can explain the divergent results from studies on CSVD and parkinsonism risk.

In our cohort of middle-aged and elderly adults, potential risk factors for late-onset of parkinsonism were investigated. Still, we cannot exclude the possibility that the subclinical vascular pathology might be associated with young-onset of parkinsonism.

In conclusion, we did not find a consistent pattern of associations between measures of subclinical vascular pathology and all-cause parkinsonism, thus our report suggest that systemic vascular pathology does not play a large role in the etiology of parkinsonism in the general population. Still, prospective studies that incorporate novel brain-imaging and have adequate sample size and follow-up may detect associations of intracranial vascular pathology with incident parkinsonism.

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Chapter 6.3

Professional occupation

ABSTRACT

Creativity in Parkinson's Disease (PD) is strongly related to dopaminergic activity and medication. We hypothesized that PD patients, including those who are in the prediagnostic phase of PD, are prone to choose highly-structured 'conventional' professional occupations and avoid highly-creative 'artistic' occupations. At baseline of the Rotterdam Study, we asked 12,147 individuals aged ≥45 years about their latest occupation, and categorized occupations according to the RIASEC model. Participants underwent baseline and follow-up (median 11 years) examinations for PD. We determined associations of artistic (versus any other occupation) and conventional (versus any other occupation) occupations with PD. Additionally, we pooled our results with a recently published case-control study ('Radboud Study'). At baseline, conventional occupations were common (n=4,356 [36%]), while artistic occupations were rare (n=137 [1%]). There were 217 PD patients, including 91 prevalent and 126 incident PD patients. The risk of PD varied substantially across occupational categories (chi-square=14.61; p=0.01). The penalized odds ratio (OR) of artistic occupations for PD was 0.19 (95% confidence interval [0.00;1.31]; p=0.11), while the OR of conventional occupations for PD was 1.23 ([0.95;1.66]; p=0.10). The direction and magnitude of ORs were similar in cross-sectional and longitudinal subsamples. Pooled ORs across the Rotterdam and Radboud studies were 0.20 ([0.08;0.52]; p<0.001) for artistic and 1.23 ([0.92;1.67]; p=0.08) for conventional occupations. The risk of PD varies substantially by choice of professional occupation. Our findings suggest that dopaminergic degeneration affects choice of occupation, which may already start in the prediagnostic phase of PD.

BACKGROUND

In Parkinson's Disease (PD) patients, low levels of dopamine and cortical dopamine receptor availability are associated with a lack of novelty seeking.[1,2] Furthermore, PD patients may show changes in personality following initiation of antiparkinson medication, progressively displaying traits such as novelty seeking.[3]Given the remarkable link between dopaminergic state and creative behavior, PD patients may be most comfortable in more structured jobs that do not require optimal dopamine levels, possibly leading to an overrepresentation in highly-structured 'conventional' occupations. In turn, PD patients may be underrepresented in 'artistic' occupations, which are strongly correlated with high-creativity traits such as 'openness to new experience'.[4]

Since clinical PD is preceded by a prediagnostic phase during which dopaminergic degeneration already leads to subtle cognitive and behavioral changes,[5,6] a distinct pattern of occupational preference may already be present in prediagnostic PD patients. Recent work from Radboudumc in Nijmegen ('Radboud Study') showed that the distribution of occupational categories indeed varied between PD patients during their prediagnostic phase and asymptomatic controls; most notably, PD patients had a reduced prevalence of artistic occupations in late-life prior to their PD diagnosis.[7]

Here, we assessed the associations of artistic and conventional occupations with PD in a population-based cohort, and we pooled estimates from this study and the recent Radboud Study to summarize all available evidence.

METHODS

Study setting, population and design

The study was embedded in the population-based Rotterdam Study.[8,9] A description of methods in the Rotterdam Study is provided directly below this paragraph. Furthermore, we pooled our results with a recently published case-control study ('Radboud Study'), which unlike the Rotterdam Study was a clinic-based, case-control study.[7] We note that there is a complementary element across both studies, each with their own strengths and weaknesses: one comprises a prospectively followed sample (no recall bias, but a low number of PD cases), the other a retrospective case-control (which may be affected by recall bias, but has a much larger number of PD cases). A description of methods in the Radboud study is provided at the end of this section.

Methods in the Rotterdam Study

The study was embedded in the Rotterdam Study, a prospective, population-based study of individuals aged 45 years and older in the well-defined Ommoord district in the city of Rotterdam comprising three subcohorts.[8,9] Baseline assessments of occupational status and prevalent parkinsonism or dementia took place in 1990 (first subcohort), 2000 (second subcohort) and 2006 (third subcohort), with a total study population of 14,926 individuals (overall response 72.0%).[8,9] Follow-up visits took place on average every 4 years, and study follow-up for incident parkinsonism was virtually complete (98%)[10] until 1 January 2015. For cross-sectional analyses, in which the outcome was prevalent PD, we used data of participants who completed the baseline interview on occupational status

and were screened for parkinsonism (n=12,147). For longitudinal analyses, in which the outcome was incident PD, we excluded individuals with prevalent parkinsonism or dementia (n=502).

During the interview on occupational status, participants who were still occupationally active were asked about their current occupation; retired or unemployed participants were asked about their last occupation. Subsequently, occupations were categorized according to the RIASEC model.[11] Participants were also extensively screened for parkinsonism and dementia using several overlapping modalities, including serial in-person examinations.

RIASEC model

To classify professional occupations, we used the RIASEC model, which is a previously validated method to classify occupations into six categories linked to personality characteristics: realistic (R), investigative (I), artistic (A), social (S), enterprising (E), and conventional (C).[11] In *Supplement 3*, we present examples and characteristics of occupations in each category. The RIASEC model has previously been employed to assess occupational preference in Asperger's Syndrome,[12] and the association of occupational choice with brain hemisphere preference.[13]

Although the influence of creativity on choice of professional occupation is likely not dichotomous, we focused on the two most contrasting RIASEC categories, i.e. 'artistic', which encompass high creativity and novelty-seeking, and 'conventional', which require high levels of structure and routines. Occupational categories,

based on professions reported by study participants at baseline, were assigned in 2016. The coder was blinded to participants' prevalent or incident PD status.

Ascertainment of parkinsonism and Parkinson's Disease

A detailed description of assessment methods is provided in **Chapter 4.1**. In short, we used four overlapping modalities to screen for potential parkinsonism: in-person examinations (on average every 4 years), in-person interviews, use of antiparkinson medication, and clinical monitoring alerts.

Of all individuals who screened positive in any of these methods, complete medical records (including letters from medical records of specialists and general practitioners) were studied and case reports were drawn up covering all potentially relevant information to establish presence and cause of parkinsonism. These case reports were evaluated by a panel led by an experienced neurologist.

Parkinsonism was defined by presence of hypo- or bradykinesia in combination with ≥1 cardinal sign (resting tremor, rigidity or postural imbalance) observed by any physician, or a clinical diagnosis of parkinsonism by a neurologist or geriatrician (if motor examination details were unavailable). PD was only diagnosed after exclusion of secondary causes, in individuals with at least one of: 1) a clinical PD diagnosis by a neurologist or geriatrician; 2) positive response to dopaminergic treatment. Individuals with parkinsonism who did not fulfill PD criteria were considered secondary parkinsonism cases. After initial diagnosis, medical records of all incident parkinsonism cases continued to be scrutinized until the end of the study period for new information that could lead to a revision of the diagnosis.

Person-time at risk of incident PD ended at the first of the following: diagnosis of incident parkinsonism (due to either PD or other causes), incident dementia, death or January 1, 2015.

Methods in the Radboud Study

A detailed description of methods used in the Radboud Study has previously been published.[7] In short, the Radboud Study was a case-control study of 693 male PD patients (defined using UK Brain Bank Criteria[14]) and 1,183 male community-dwelling controls. Participants were asked about their most recent and first ever occupation before parkinsonism onset. Similar as in the Rotterdam Study, occupations were categorized according to the RIASEC model.[11] We only used data on the most recent occupation for meta-analyses of the Rotterdam and Radboud studies, since first ever occupation was not obtained from Rotterdam Study participants.

Statistical analysis

We separately analyzed the associations of artistic occupations (versus any other occupation) and conventional occupations (versus any other occupation) with PD. We used Firth's penalized logistic regression models to account for the presence of statistical separation in our models, with adjustment for age, sex, education, subcohort and time at risk of PD (in longitudinal analyses).[15,16] This method helps to avoid overestimation of the difference in PD risk across occupational categories. We constructed penalized regression models for ever-PD (i.e., prevalent or incident PD) to meta-analyse cross-sectional and longitudinal data,

and pooled results with the Radboud Study using inverse-variance weighting or, in case of statistical separation, Mantel-Haenszel's method.[7]

In post-hoc sensitivity analyses on the link between artistic occupations and PD, we repeated the main analysis after simulating that 1 or 2 randomly selected individuals with an artistic occupation in the Rotterdam Study would have had PD. We performed 1,000 simulations for each scenario, and calculated mean odds ratios of these simulations in the Rotterdam Study as well as corresponding pooled odds ratios across the Rotterdam and Radboud studies.

RESULTS

Baseline characteristics of the study population are shown in *Table 1*. Conventional occupations were common (4,356 [36%]), while artistic occupations were rare (n=137 [1%]) in this population. 65% of individuals with conventional occupations were women, compared to only 27% of individuals with artistic occupations ($p<0.001$). Among those with artistic occupations, 49% were still employed at study enrollment, which was distinctly higher than the 29% of individuals with a conventional occupation who were still employed ($p<0.001$). Of individuals with artistic or conventional occupations who were not professionally active at baseline, most had retired, while <3% was unemployed (*Supplement 2*). The most common artistic occupation was musician, while the most common conventional occupation was shop employee. The median duration of latest occupation, which was available in 2,373 individuals, was 12 years for conventional and 31 years for artistic occupations.

There were 123 patients with prevalent parkinsonism, of whom 91 had prevalent PD. Thirty-two prevalent PD patients (35%) and 4,324 controls (35%) had held a conventional occupation, while 0 prevalent PD patients and 137 controls (1%) had an artistic occupation. During follow-up, 266 individuals were diagnosed with incident parkinsonism, including 126 with incident PD. Fifty-one (41%) incident PD patients had a conventional occupation compared to 4,194 controls (36%). No incident PD patients and 132 controls (1%) had an artistic occupation. The full distribution of occupational categories by ever-PD status is presented in *Supplement 1*.

Table 1 | Characteristics of study population, by occupation.

Characteristic	R	I	A	S	E	C	Total	*P [A vs. C]**
Number of individuals	3679	293	137	1771	1911	4356	12147	
Age, median [IQR], years	68 [15]	62 [12]	64 [13]	62 [10]	64 [12]	64 [12]	65 [13]	*0.98*
Female sex [%]	1776 [48]	59 [20]	37 [27]	1411 [80]	678 [35]	2850 [65]	6811 [56]	*<0.001*
Intermediate or higher education [%]	740 [21]	98 [71]	46 [55]	487 [44]	612 [40]	1403 [36]	3386 [33]	*0.08*
Smoking at baseline [%]	549 [15]	19 [6]	14 [10]	141 [8]	230 [12]	491 [11]	1444 [12]	*0.38*
Employed at baseline [%]	731 [20]	141 [48]	67 [49]	701 [40]	685 [36]	1182 [27]	3507 [29]	*<0.001*

*R, Realistic. I, Investigative. A, Artistic. S, Social. E, Enterprising. C, Conventional. IQR, interquartile range. %, percentage of individuals with complete characteristics data. Intermediate or higher education includes intermediate vocational education or higher general education higher vocational education or university. *P values for difference between individuals with artistic vs. conventional occupations. Comparisons on age and sex were adjusted for each other; comparisons on education, smoking and employment were adjusted for age and sex.*

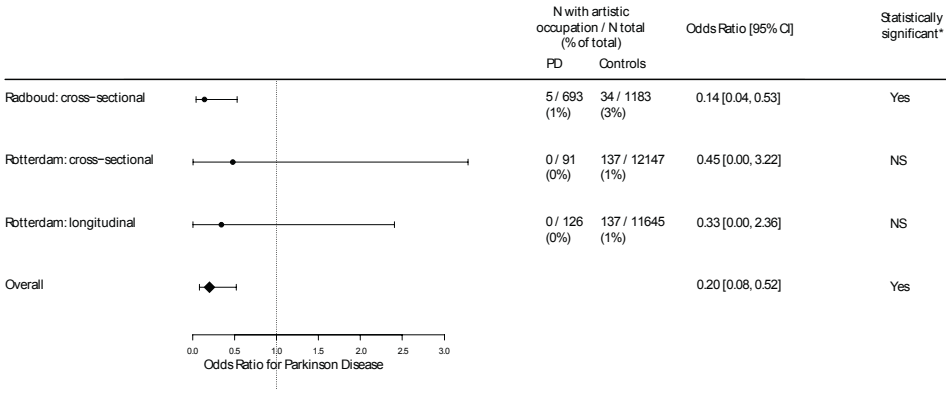
In combined cross-sectional and longitudinal analyses, the risk of ever-PD varied substantially across occupational categories (chi-square=14.61; p=0.01). Individuals with conventional occupations had an odds ratio of 1.26 for PD (95% confidence interval [0.95; 1.66]; p=0.10), whereas individuals with artistic occupations had an odds ratio of 0.19 for PD (95% confidence interval [0.00; 1.31]; p=0.11). In sensitivity analyses, exclusion of prevalent PD patients who were still employed at study enrollment (n=19) had virtually no effect on odds ratios of conventional professions (OR=1.32 [0.98; 1.77]; p=0.06) or artistic professions (OR=0.21 [0.00; 1.48]; p=0.15) for PD. Additional adjustment of the main analyses for smoking and employment status did somewhat attenuate the odds ratios of conventional (OR=1.19 [0.88; 1.77]; p=0.26) and artistic (OR=0.28 [0.00; 2.00]; p=0.26) occupations for PD.

In the Radboud Study, the risk of PD also varied distinctly across occupational categories (chi-square=41.41; p<0.001). Pooled odds ratios for PD across the Rotterdam and Radboud studies were 1.23 ([0.92; 1.67]; p=0.08) for conventional and 0.20 ([0.08; 0.52]; p<0.001) for artistic occupations. Separate odds ratios for cross-sectional analyses, longitudinal analyses, as well as odds ratios of the Radboud Study are presented in *Figure 1*.

While the protective effect of artistic occupation on risk of PD in the Rotterdam Study incrementally attenuated if 1 or 2 individuals with artistic occupation would have had PD, the overall association would have remained statistically robust in a pooled analysis of the Rotterdam and Radboud studies (*Table 2*).

Figure 1 | Odds ratios of artistic and conventional occupations for Parkinson's Disease.

A. Artistic occupations



B. Conventional occupations

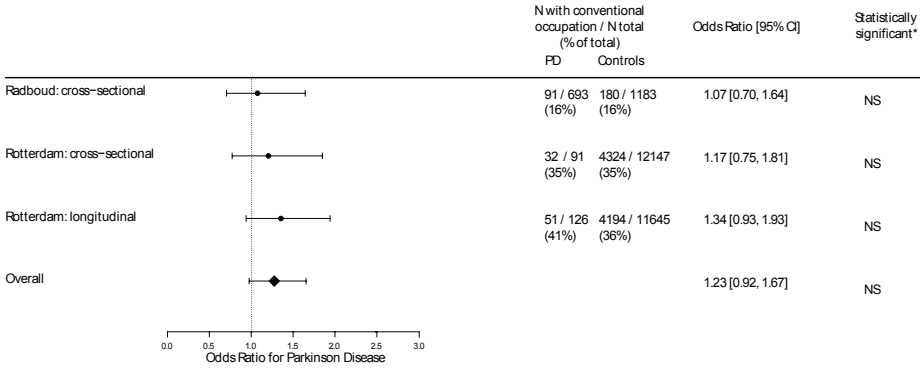


*Figure 1A. Artistic occupations. Odds ratio of artistic vs. any other occupation for Parkinson's Disease. Figure 1B. Conventional occupations. Odds ratio of conventional vs. any other occupation for Parkinson's Disease. N, number. PD, Parkinson's Disease. CI, 95% confidence interval. NS, not statistically significant at α=0.05. *Not statistically significant at α=0.05. Analyses in the Rotterdam Study were adjusted for age, sex, education and study subcohort. Analyses in the males-only Radboud Study were adjusted for age.*

Table 2 | Sensitivity analyses: simulation of different Parkinson's Disease distribution by artistic occupation.

N artistic individuals with PD	Odds ratio [CI] for PD in Rotterdam Study	Odds ratio [CI] for PD in pooled analysis
Observed		
0	0.19 [0.00; 1.31]	0.20 [0.08; 0.52]
Simulated		
1	0.57 [0.06; 2.11]	0.28 [0.12; 0.64]
2	0.97 [0.20; 2.83]	0.34 [0.15; 0.75]

N, number. PD, Parkinson's Disease. CI, 95% confidence interval.

DISCUSSION

In this population-based sample, choice of professional occupation in middle-aged and older adults was associated with the risk of PD. This observation is in line with the recent Radboud Study.[7] Taken together, these studies specifically show that individuals with highly-creative artistic occupations have a reduced risk of PD, while individuals with highly-structured conventional occupations may have an increased risk of PD, although not statistically significant.

As far as we know, farming is the sole occupational group that has consistently been demonstrated to be associated with the risk of PD.[17] In the current study, however, we used professional occupation as a proxy of personality. Individuals with artistic occupations have the most openness to new experiences, while those with conventional occupations have the least.[4] In turn, dopamine levels and cortical D2 receptor availability are strongly associated with creativity in PD,(1-3) and PD patients who are being treated with dopaminergic medication can newly develop artistic expressions that they had not previously shown.(4-6) Given the

strong association between dopaminergic function and creative behavior in PD, we believe that the inverse association of artistic occupations with the risk of PD reflects a larger degree of dopaminergic degeneration in (prediagnostic) PD patients. Our study extends on these findings by establishing an association between choice of professional occupation and the risk of PD.

We consider three alternative hypotheses that may explain our observations. First, the choice of professional occupation may be associated with addictive personality traits that predispose to substance use. For instance, individuals with artistic professions may have a higher prevalence of smoking, which may by itself affect (namely: lower) the risk of PD.[17] We note, however, that causality of the inverse association of addictive substances in general, and of smoking in particular, with the risk of PD remains highly contentious.[18] Second, we cannot rule out that individuals with artistic occupations have a lower risk of PD because of nondopaminergic pathways, although we are unaware of consistent empirical evidence for a biological link of artistic or conventional occupations with serotonergic or noradrenergic pathways. Third, high early-life dopamine levels may increase the threshold for clinical parkinsonism to occur (higher dopamine reserve), even in the presence of PD pathology, similar to the observation that high education is associated with relative preservation of cognitive abilities in PD patients with cortical β-amyloid pathology.[19]

The main strength of our study is its population-based design, which ensures a representative sample of PD patients and controls in the community. The main limitation of our study is the small number of participants with an artistic occupation (n=137). Consequently, we were underpowered to restrict our analyses to incident PD patients. While the overall response figure of the study was high (72%) and ORs of the associations were similar in cross-sectional and

longitudinal subsamples, we cannot completely rule out that PD patients with artistic occupations disproportionally more often changed their occupation due to onset of parkinsonism -or even due to prediagnostic subtle motor features- than did PD patients with other occupations. Also, while the association of artistic occupations with PD in our study is direction-consistent and similar in magnitude as in the recent Radboud Study,[7] caution is warranted in interpreting the very low odds ratio. Had a few more individuals with artistic occupations been diagnosed with PD in the Rotterdam Study, the association would have attenuated in the Rotterdam Study but would have remained robust in the pooled analysis.

In this study, the confidence interval of the odds ratio of conventional occupations for PD overlapped, even in the pooled analysis. This either suggests that there is no association between conventional occupations and PD or, alternatively, that this study was statistically insufficiently powered to detect a true association with a modest effect size that may be significant from a clinical perspective (i.e., odds ratio~1.2). To robustly determine whether there is an association between conventional occupations and PD, future studies with a larger sample size are warranted." Future studies should also focus on individuals with investigative occupations, which are characterized by traits that are often observed in PD patients such as precision, reason and independent work.

In conclusion, this population-based study extends a recent case-control study, and again suggests that the risk of PD varies substantially by choice of professional occupation in midlife. While we interpret our findings cautiously, this converging evidence suggests that dopaminergic degeneration affects choice of occupation, which may already start in the prediagnostic phase of PD. Further large studies are warranted to robustly quantify the effect size of the associations of artistic and conventional occupations with incident PD.

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Chapter 7

Parkinson's Disease: prodromal changes

Long before rigidity actually develops, patients have significant difficulty performing ordinary activities.

Charcot J-M 1872. De la paralysie agitante. In Oeuvres Complètes (t 1) Leçons sur les maladies du système nerveux, pp. 155–188 A Delahaye, Paris. [Translated from French to English]

Chapter 7.1

Trajectories

ABSTRACT

At the time of clinical diagnosis, patients with Parkinson's Disease (PD) already have a wide range of motor and non-motor features that affect their daily functioning. However, the temporal sequence of occurrence of these features remains largely unknown. We studied trajectories of daily functioning and motor and non-motor features in the 23 years preceding PD diagnosis by performing a nested case-control study within the prospective Rotterdam Study. Between 1990 and 2013, we repeatedly performed standardized assessments of daily functioning (Stanford Health Assessment Questionnaire, Lawton Instrumental Activities of Daily Living scale), potential prediagnostic motor (hypo- and bradykinesia, tremor, rigidity, postural imbalance, postural abnormalities) and non-motor features of PD, including cognition (Mini-Mental State Exam, Stroop test, Letter-Digit-Substitution Test, Word Fluency Test), mood (Center for Epidemiological Studies-Depression Scale, Hamilton Anxiety and Depression Scale), and autonomic function (blood pressure, laxative use). In addition, the cohort was followed-up for the onset of clinical PD using several overlapping modalities, including repeated in-person examinations as well as complete access to medical records and specialist letters of study participants. During follow-up, 109 individuals were diagnosed with PD, and each case was matched to 10 controls based on age and sex (total n=1,199). Subsequently, we compared prediagnostic trajectories of daily functioning and other features between PD cases and controls. From seven years before diagnosis onwards, prediagnostic PD cases more commonly had problems in instrumental activities of daily functioning, and more frequently showed signs of movement poverty and slowness, tremor and subtle cognitive deficits. In the last five years, PD cases developed additional motor features (postural imbalance, rigidity, and postural abnormalities) and increasingly reported problems in basic daily activities.

PD cases also increasingly reported anxiety symptoms, depressive symptoms, and use of laxatives throughout study follow-up, although differences with controls only became statistically significant in the last years before diagnosis. In conclusion, in prediagnostic PD patients, impairments in instrumental daily activities, which require both motor and non-motor skills, predate difficulties in more physically oriented daily activities.

BACKGROUND

Although primarily characterized by a set of motor symptoms known as parkinsonism, early clinical PD patients may present with various features that affect daily functioning well beyond impaired motor function, such as mild cognitive impairment, depressive symptoms, and mild autonomic dysfunction.[1,2] To date, no therapies have been shown to modify disease progression in patients with PD, which may be a consequence of the advanced stage of pathology that early clinical PD patients already have.[3] Therefore, there is growing interest in defining earlier stages of the disease.

Over seven decades ago, it was already recognized that a proportion of patients who went on to be diagnosed with PD had prodromal symptoms.[4] This observation was later corroborated by a case study of professional soccer player Ray Kennedy, who had presented with prodromal symptoms years before PD diagnosis, including motor symptoms seven years before diagnosis.[5] A Dutch case-control study subsequently identified central nervous system, psychologic, musculoskeletal, and autonomic symptoms in prediagnostic PD patients.[6] More recently, a registry-based study showed that in the ten years preceding clinical diagnosis a range of motor and non-motor features become increasingly prevalent in PD patients.[7] However, to date, no study has been published on long-term trajectories of prediagnostic features using data that were repeatedly and consistently assessed in both PD patients and controls. Furthermore, patterns of deterioration in various motor, limbic, autonomic, and cognitive features of PD have not yet been explored systematically. Insight into these trajectories, and their combined effects on daily functioning, could possibly aid in earlier diagnosis of PD and contribute to the identification of individuals who would benefit from early symptomatic treatment.

Moreover, it may inform clinical studies on which individuals may be most suitable for inclusion in neuroprotective trials.

Within the prospective Rotterdam Study, we used repeated assessments to investigate differences in prediagnostic trajectories of PD patients and controls.

METHODS

Setting

The study was embedded in the Rotterdam Study, a large, prospective, population-based cohort study in the Netherlands.[8,9] The study was initiated in 1990, inviting all inhabitants of Ommoord aged ≥55 years. 7,983 participants (78%) agreed to participate. At baseline, participants were screened for parkinsonism and dementia. For this report, we excluded individuals with prevalent parkinsonism or dementia or an unknown status of either, leaving 6,456 individuals at risk of PD.

We monitored participants for the development of PD from baseline until first of: parkinsonism, dementia, death or January 1st, 2013. Until 2013, the study has had a total of five visits, including four follow-up visits between: 1993-1995, 1997-1999, 2002-2004, and 2009-2011. At each visit, participants undergo home interviews and medical examinations at the research center. We studied daily functioning as well as motor and non-motor features that were measured at least at three different visits, except for anxiety, which was only measured at two visits. An overview of the assessed features is presented in *Table 1* and additional details on assessment moments are presented in *Online Supplementary Table 1*. Study follow-up for PD was virtually complete.

Study design and study population

This study consists of a matched nested case-control sample from the Rotterdam Study. The overall age- and sex-adjusted incidence rate of PD in the Rotterdam Study is 1.5 per 1000 person-years,[10] and age-specific incidence rates are somewhat higher than in most other population-based studies.[11] 59% of participants in this cohort are female.[12]

All participants diagnosed with PD during follow-up were included as cases, if they met the following criteria: they were non-demented and had no parkinsonism at the baseline visit; they were not diagnosed with dementia before PD diagnosis; they were not diagnosed with parkinsonism due to other causes; and they participated at the visit before PD diagnosis. Participants used as controls had to have the following characteristics: they were non-demented and had no PD at baseline visit; they were free of parkinsonism and dementia at the time of diagnosis of the matched PD case; they participated at the visit before diagnosis of the matched PD case; they were matched to a PD case by age (± 3 years) and sex. We matched 10 controls for every PD case at follow-up, resulting in inclusion of 109 PD cases and 1,090 controls (total n=1,199). Due to missing data on some features, numbers of participants may vary across individual analyses.

Table 1 (part I) | Overview of potential prediagnostic features of Parkinson's Disease in the Rotterdam Study.

Group	Feature	Test(s)	Interpretation
Motor features		Arm swing (L/R)	0. Normal; 2. Reduced
		Gait	0. Normal; 1. Shuffling / small steps; 2. Propulsion + festination
	Hypo- and/or bradykinesia	General impression of hypo- and bradykinesia	0. Normal; 1. Less spontaneous movement; 2. Clear movement poverty or slowness
		Finger tapping (L/R)	0. Normal; 1. Somewhat slowed down /lower amplitude; 2. Slowed down
	Tremor	Resting tremor (L/R) Intention tremor (L/R) Positional tremor (L/R)	0. No; 1. Doubtful; 2. Yes
	Rigidity	Tone arm (L/R)	0. Normal; 1. Raised
	Postural balance	Maintain standing balance in steady stance in response to external perturbation	0. Normal; 1. Doubtful; 2. Impaired
	Postural abnormalities	General impression of posture	0. Fitting age; 1. Head/neck/arms flexed; 2. Kyphosis and arms/legs flexed
	Falling	Questionnaire / Self-reported fall in the previous 12 months	0. No; 1. Yes

Table 1 (part II) | Overview of potential prediagnostic features of Parkinson's Disease in the Rotterdam Study.

Group	Feature	Test(s)	Interpretation
Non-motor features	Cognition: objective	Mini-Mental State Exam (MMSE)	0-30 points
		Stroop test tasks 1-3	N seconds needed to finish the task
		Letter-Digit-Substitution Test	N correct letter-digit combinations in 60 seconds
		Word Fluency Test	N animals correctly named in 60 seconds
	Cognition: subjective	Memory complaints (3 items)	0. No; 1. Yes
	Depressive symptoms	CESD depression	0-60 points
	Anxiety symptoms	HADS anxiety	0-21 points
	Hypotension	Systolic & diastolic blood pressure	Continuous scale (mm Hg)
	Constipation	Self-reported laxative use	0. No; 1. Yes
Daily functioning	BADL	Stanford	8 component scores & overall score
	IADL	IADL scale	8 component scores & overall score

L, left. R, right. N, number of.
Center for Epidemiological Studies - Depression Scale (CES-D; 20 items).
HADS, Hamilton Anxiety and Depression Scale (anxiety subscale).
BADL, Basic Activities of Daily Living.
Stanford, Stanford Health Assessment Questionnaire (20 items).
IADL, Instrumental Activities of Daily Living.
IADL scale, Lawton and Brody scale (8 items).

Ascertainment of parkinsonism and Parkinson's Disease

A detailed description of assessment methods is provided in **Chapter 4.1**. In short, we used four overlapping modalities to screen for potential parkinsonism: in-person examinations (on average every 4 years), in-person interviews, use of antiparkinson medication, and clinical monitoring alerts.

Of all individuals who screened positive in any of these methods, complete medical records (including letters from medical records of specialists and general practitioners) were studied and case reports were drawn up covering all potentially relevant information to establish presence and cause of parkinsonism. These case reports were evaluated by a panel led by an experienced neurologist.

Parkinsonism was defined by presence of hypo- or bradykinesia in combination with ≥1 cardinal sign (resting tremor, rigidity or postural imbalance) observed by any physician, or a clinical diagnosis of parkinsonism by a neurologist or geriatrician (if motor examination details were unavailable). PD was only diagnosed after exclusion of secondary causes, in individuals with at least one of: 1) a clinical PD diagnosis by a neurologist or geriatrician; 2) positive response to dopaminergic treatment. Individuals with parkinsonism who did not fulfill PD criteria were considered secondary parkinsonism cases. After initial diagnosis, medical records of all incident parkinsonism cases continued to be scrutinized until the end of the study period for new information that could lead to a revision of the diagnosis.

Person-time at risk for incident PD ended at the first of the following: diagnosis of incident parkinsonism (due to either PD or other causes), incident dementia, death or January 1st, 2013.

Ascertainment of dementia

Participants were screened for dementia at baseline and follow-up examinations using a three-step protocol [13], comprising two brief tests of cognition (Mini-Mental State Examination [MMSE] and Geriatric Mental State schedule [GMS] organic level.[14,15]) Individuals with positive screen results then underwent the Cambridge Examination for Mental Disorders of the Elderly.[16] Additional information was obtained from in-person examination by a neuropsychologist, clinical monitoring and neuro-imaging. A consensus panel, led by an experienced neurologist, decided on the final diagnosis in accordance with standard criteria using the DSM-III-R criteria for dementia.

Prediagnostic features investigated: daily functioning, motor features, and non-motor features

We considered various putative features of PD that have been implicated during the prediagnostic phase of the disease. These features were grouped into daily functioning, motor features, and non-motor features. This is also the order that we use to describe ascertainment methods and present results.

Daily functioning

Functioning in Basic Activities of Daily Living (BADL) was assessed using the disability index of the Stanford Health Assessment Questionnaire.[17] The questionnaire consists of 20 items constituting eight components: activities, arising, dressing and grooming, eating, hygiene, grip, reach, and walking. In our study, two

of three items of eating (ability to cut meat and drink a glass of milk) were combined into one. All items could be scored from 0 to 3, with higher scores reflecting worse ability: 0=no difficulty, 1=some difficulty, 2=much difficulty, and 3=unable to. Component scores were calculated as the highest scored item belonging to the respective component. Subsequently, BADL was calculated as the sum of the eight components.

Functioning in Instrumental Activities of Daily Living (IADL) was assessed using the Instrumental Activities of Daily Living scale.[18] The IADL-scale consists of eight items: shopping, washing, travelling on your own, finance management, phoning, medication use, housekeeping, and meal preparation. Similar to BADL, items were coded from 0 to 3, with higher scores reflecting worse ability. For IADL, items scored as non-applicable were imputed using the mean of five imputations, based on age, sex, scores on BADL items, and scores on other IADL items. Imputation of non-applicable values has been suggested and implemented by previous studies to prevent loss of data.[19,20] Imputations were performed separately for each study visit, with 5.3% or less of variables imputed per visit. Subsequently, IADL was calculated by summing the eight items.

Motor features

At baseline and follow-up visits, participants were screened for parkinsonian signs by research nurses using standardized methods. At baseline and repeatedly throughout the study period, these research nurses were trained by neurologists specialized in movement disorders to ensure consistent data on objective motor features. Importantly, these assessments were not used for diagnostic purposes, i.e.

they were not considered in the process of establishing a diagnosis of parkinsonism.

We assessed the following motor features: any tremor, any hypo- and bradykinesia, rigidity, postural abnormalities, and postural balance. For features assessed on both sides, the highest score of both sides was used. Separate tremor observations (0=absent, 1=doubtful, 2=present) on both sides included resting tremor, positional tremor, and intention tremor. We defined a composite tremor score ("any tremor") using the highest observation for any of the separate items on both sides. Of hypo- and bradykinesia observations, we assessed finger tapping on both sides and used the highest observation (0=absent, 1=doubtful, 2=slowed down or reduced). In addition, we classified gait (0=normal, 1=shuffling and small steps, 2=propulsion and festination), arm swing (0=normal, 2=reduced), and general impression of hypo- and bradykinesia (0=absent, 1=doubtful, 2=present). A composite score for "any hypo- or bradykinesia" was defined as the highest score for any separate hypo- or bradykinesia item. For rigidity, tone in both arms (0=normal, 1=elevated) was assessed. Postural balance was classified as 0=normal, 1=slightly disturbed or 2=absent. Posture was classified as 0=fitting age, 1=head/neck/arms flexed or 2=kyphosis and arms/legs flexed.

In addition, at each follow-up visit, individuals were asked whether they had fallen in the previous 12 months.

Non-motor features

We assessed cognitive functioning objectively using the MMSE,[14] Stroop color word test (comprising three tasks),[21] Letter Digit Substitution Test (LDST),[22] and Word

Fluency Test (WFT).[23] The abbreviated Stroop test consists of three subtasks in which the participant is shown a (colored) card with 40 items that have to be named.[21] In trial one, the participants are asked to name the printed words; in the second trial the participants are asked to name the printed colors; in the third trial the participants are asked to name the color in which each color-name is printed. For each trial, the time to complete the task was used as the outcome, which implies that a higher score indicates a worse performance. The LDST is a modified version of the Symbol Digit Modalities Test[22] and asks the participants to make as many letter-digit combinations as possible in 60 seconds, following an example that shows correct combinations. In the WFT, participants were asked to name as many animals as possible within 60 seconds.[23] For both the WFT and LDST the number of correct answers was used as the outcome.

Subjective memory complaints were assessed using three questions, which could be answered by yes or no. These questions were: "Do you have more trouble remembering things than before?"; "Does it happen more often that you are on your way to do something and forget what you wanted to do?"; and "Do you more often have trouble finding words during a conversation?" Depressive symptoms were assessed during a home interview using the Dutch version of the original Center for Epidemiological Studies Depression Scale (CES-D). The CES-D is a 20 item self-reported measure of symptoms, scored on a scale from 0 to 3.[24] Anxiety symptoms were assessed using the anxiety part of the Hamilton Anxiety and Depression Scale.[25]

Blood pressure was measured by using a random-zero sphygmomanometer at the right brachial artery in sitting position after a 5-minute rest. The average of two consecutive BP measurements was used. By means of in-person interviews we obtained information on the use of blood-pressure lowering medication (ATC

codes C02, C03, C07, C08, and C09) as well as the use of laxative medication, which we used as a proxy for constipation since no data on stool frequency was available.

Statistical analysis

We used extended mixed models to investigate differences in trajectories of decline in daily functioning, motor features, and non-motor features, and with incident PD. In subanalyses, we explored which BADL-component and IADL-item was first to decline, and separately we used different thresholds for BADL and IADL scores to assess the proportion of prediagnostic cases and controls with any (score $\geq$1), moderate/severe (score $\geq$9), or severe ($\geq$17) impairment.

Similar to a previous study,[26] we used a link function with quadratic splines to capture the distribution and account for ceiling effects of dependent variables with ceilings that were not dichotomous. To properly adhere to the distribution of dependent variables, we used the Bayesian information criterion (BIC) to estimate the optimal number and position of splines in a basic model including age, sex, their interaction terms with time, age*time^2, time itself, time^2, and time^3. For dichotomous or continuous dependent variables, a linear link function was used. Random intercepts and random slopes over time were included.

Time was calculated from clinical diagnosis of parkinsonism for PD cases, so that time=0 corresponds to time of clinical diagnosis. For controls, we subtracted time to PD diagnosis of the matched PD case from the follow-up of the control so that at time=0, follow-up time for controls equals that of their matched PD case.

We investigated up to cubic associations of incident PD with motor and non-motor features over time (i.e. interactions with time, time^2, and time^3), adjusting for any

non-time dependent effect of incident PD. For visualization of trajectories in motor and non-motor features, models including all interaction terms of PD up to time^3 were used. All analyses were adjusted for the basic model of age, sex, education, their interactions with time, age*time^2, time itself, time^2, and time^3. Additionally, analyses on systolic and diastolic blood pressure were adjusted for use of blood pressure lowering drugs at the respective visits. We present trajectories of the last 14 years before (matched) diagnosis visually, since differences between PD cases and controls generally occurred during that period.

Since dementia before parkinsonism onset is no longer an exclusion criterion for PD in the MDS diagnostic criteria,[27] we explored how prediagnostic IADL, BADL, hypo- or bradykinesia and MMSE trajectories would be affected by the addition of parkinsonism patients with a preceding diagnosis of dementia (but without a clear secondary cause of parkinsonism) to the PD case group. In separate sensitivity analyses, we restricted trajectory analyses of IADL, BADL, hypo- or bradykinesia and MMSE to PD patients who fulfilled a stricter case definition: their parkinsonism had to be diagnosed by a neurologist or geriatrician and they had to have had a positive response to dopaminergic medication.

We investigated rank ordering of motor features by subtracting the trajectories for these features of controls from the trajectories of PD cases and plotted these in the same figure. Hence, these trajectories reflect the additional decline in people that develop incident PD above decline in people not developing PD. After initial analyses showed that hypo- and bradykinetic features were highly frequent in prediagnostic PD patients, we also investigated rank ordering of these features separately. For non-motor features, instrument scales were highly discordant; therefore, we refrained from comparing rank ordering of these features.

Since the spline link function estimates used to transform the dependent variables vary across analyses, effect sizes of associations cannot be readily interpreted. Hence, only significance of associations ($p<0.05$) is reported. We used the "WaldMult" function in R using nominal thresholds of statistical significance ($p<0.05$) to determine when trajectories differed significantly between PD cases and controls. All analyses were performed using R version 3.1.2.

RESULTS

Population characteristics

The average (matched) age at clinical PD diagnosis was 78 years (standard deviation seven years) and 56 PD cases (51%) were women. The mean Hoehn & Yahr score at time of clinical diagnosis was 1.6. Numbers of participants included per visit prior to PD diagnosis are presented in Table *2*. As shown in *Supplementary Table 2*, selected controls had a slightly lower prevalence of hypo- and bradykinetic signs, tremor and postural imbalance than individuals who were not selected as controls.

Daily functioning

Problems in Instrumental IADL became more evident among PD cases than controls as early as 7.2 years before clinical diagnosis.(*Figure 1A*) This was followed by increasing difficulties in more physically driven BADL at 5.4 years.(*Figure 1B*) Of IADL domains, earliest differences were found for problems in travelling.(7.7 years; *Figure*

Table 2 | Number of incident Parkinson's Disease cases and controls participating across visits.

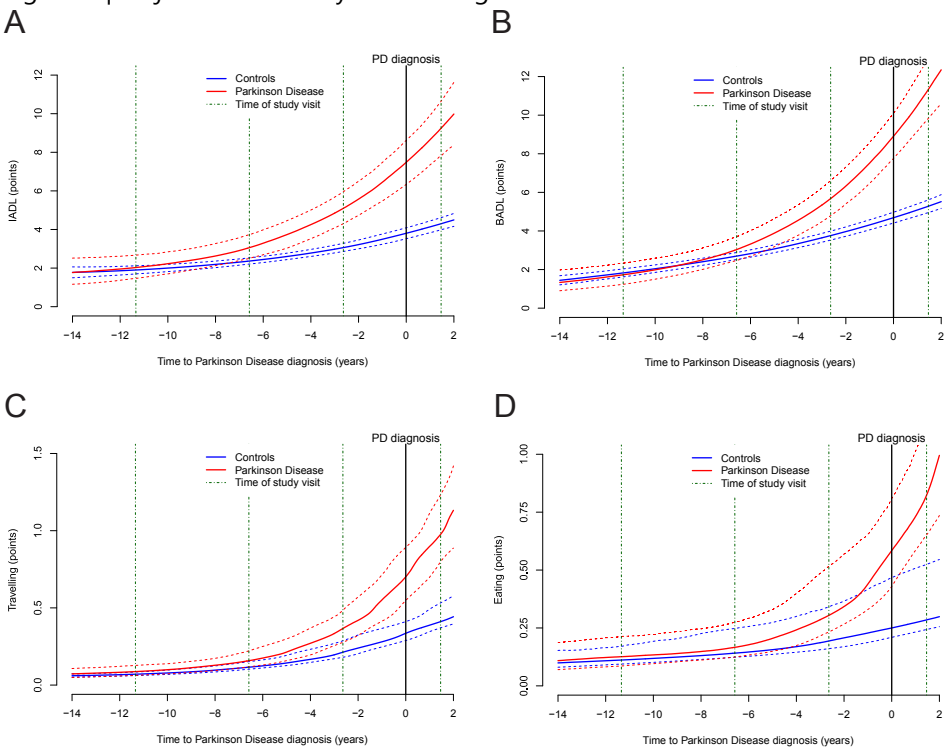
Visit	Time to PD diagnosis, years (SD)	Cases, n	Controls, n	Visit	Time to Parkinson's Disease, years (SD)	Cases, n	Controls, n
-4	15.4 (2.4)	31	302	-4	15.4 (2.4)	31	302
-3	-11.3 (2.8)	45	451	-3	-11.3 (2.8)	45	451
-2	-6.6 (2.3)	79	760	-2	-6.6 (2.3)	79	760
-1	-2.6 (1.8)	103	1,004	-1	-2.6 (1.8)	103	1,004
1	1.5 (1.9)	70	944	1	1.5 (1.9)	70	944

PD, Parkinson's Disease. Negative visits represent visits before Parkinson's Disease diagnosis, while the positive visit represents the visit after diagnosis. n, number of individuals. SD, standard deviation.

1C) Of BADL domains, problems in eating were earliest to differ between prediagnostic PD cases and controls.(5.7 years; *Figure 1D*)

An increasing proportion of prediagnostic PD patients had any IADL impairment or any BADL impairment in the last years before clinical diagnosis, but the incidence of any IADL impairment and BADL impairment also rose among controls, albeit to slightly lower levels.(*Online Supplementary Figure 4A* and *4B*) When thresholds for impairment were increased, the relative difference between PD cases and controls became more evident for both BADL and IADL impairment, but the proportion of prediagnostic PD patients that was impaired also decreased for each.(*Online Supplementary Figure 4C, 4D, 4E* and *4F*) Differences between PD cases and controls became statistically significant at the following timepoints: any IADL impairment -6.3 years, any BADL impairment -3.3 years, moderate or severe IADL impairment -5.6 years, moderate or severe BADL impairment -5.8 years, severe IADL impairment -3.7 years, severe BADL impairment -3.1 years.

Figure 1 | Trajectories of daily functioning.



Y-axis denotes average score for Parkinson's Disease patients and controls separately. Figure 1A: IADL, instrumental Activities of Daily Living. 1B: BADL, basic Activities of Daily Living. 1C: Travelling. 1D: Eating. Higher BADL and IADL scores correspond to higher impairment. PD, Parkinson's Disease. For each group, correspondingly colored dotted lines reflect confidence intervals.

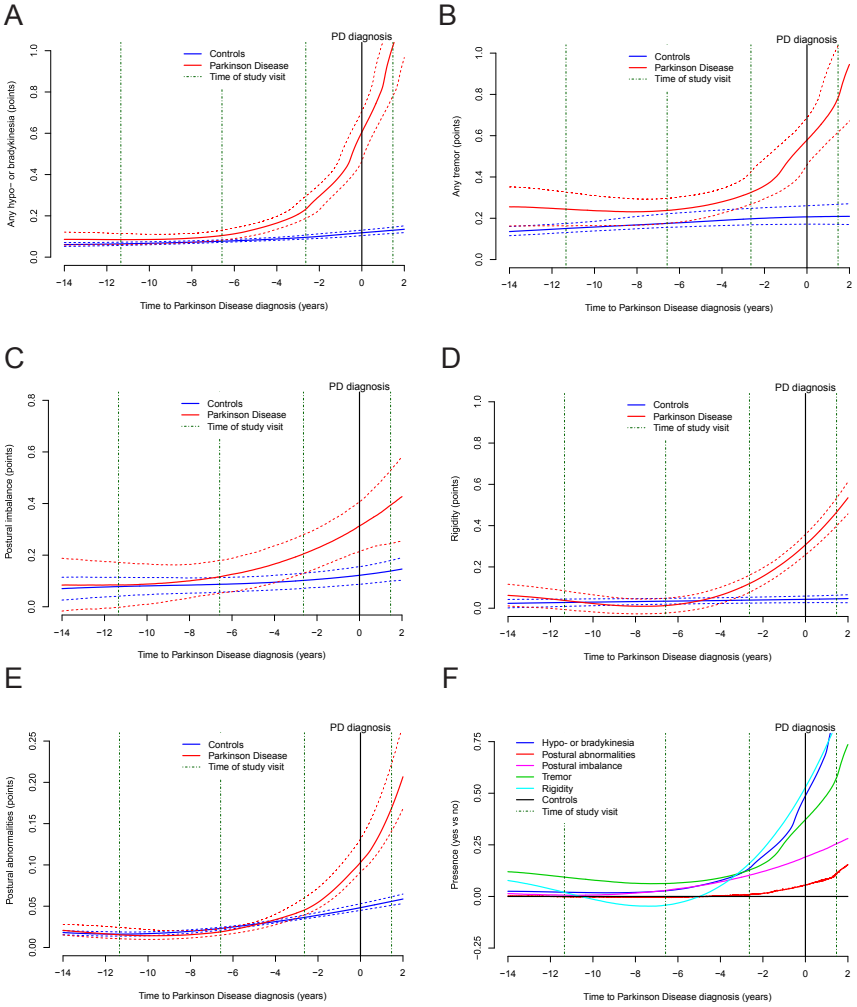
Motor features

PD cases increasingly showed hypo- and bradykinetic signs throughout study follow-up, with a statistically significant overall difference compared to controls from 7.5 years before diagnosis onwards (*Figure 2A*). Although tremor appeared to be more common in PD cases as early as 14 years before PD diagnosis, differences with controls were only significant in the last 6.1 years (*Figure 2B*). The presence of

objective postural imbalance increased more rapidly in PD cases than in controls in the last ten years before diagnosis, with significant differences in the last 3.8 years. (*Figure 2C*) Around three years before PD diagnosis, the presence of rigidity and postural abnormalities became more frequent in PD cases than in controls.(3.3 and 2.8 years; *Figures 2D and 2E*) PD cases more frequently reported falling than controls as early as fourteen years before diagnosis, but differences only became statistically significant 1.7 years before clinical diagnosis. At clinical diagnosis, rigidity, tremor and hypo- or bradykinesia were the most frequent features in PD cases.(*Figure 2F*)

Of hypo- and bradykinetic features, PD cases first more frequently had slowed finger tapping than controls throughout the entire prediagnostic phase, already from 15.8 ears before clinical diagnosis onwards.(*Online Supplementary Figure 1A*) PD cases increasingly showed a reduced arm swing, with significant differences in the last 8.6 years.(*Online Supplementary Figure 1B*) In the last four years, PD cases also more frequently presented a general impression of movement poverty or slowness (*Online Supplementary Figure 1C*), and deteriorating gait followed in the last 4.5 years.(*Online Supplementary Figure 1D*) At clinical diagnosis, reduced arm swing and slowed finger tapping were the most common hypo- and bradykinetic feature among PD cases.(*Online Supplementary Figure 1E*)

Figure 2 | Trajectories of motor features.



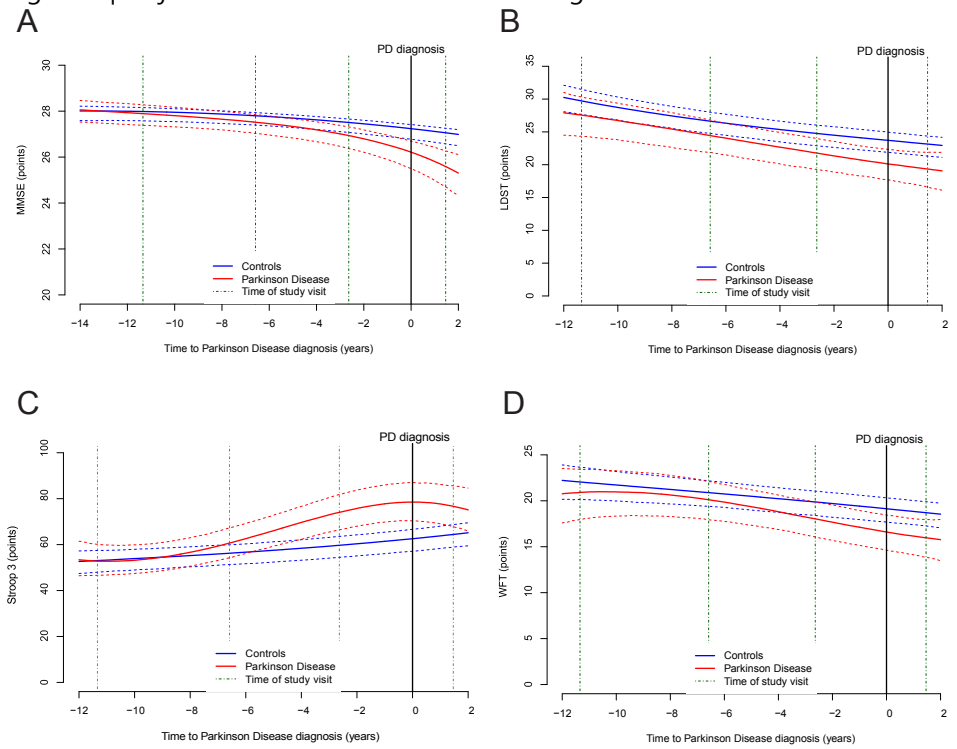
Trajectories of motor symptoms of Parkinson's Disease cases and controls. Solid lines represent mean values and dotted lines the 95% confidence intervals. Figure 2A: Any Hypo-/bradykinesia. 2B: Any tremor. 2C: Postural imbalance. 2D: Rigidity. 2E: Postural abnormalities. Figures 2A-E: Average scores were calculated on a 0-2 scale, with 0 typically denoting normal, 1 doubtful, and 2 abnormal. PD, Parkinson's Disease. Figure 2F: Trajectories of all motor symptoms in one graph. Trajectories are created by subtracting trajectories of controls from those of Parkinson's Disease cases. Hence, y=0 reflects the trajectory of controls. Of note, rigidity scores were doubled to facilitate comparison with the other motor features. For each group, colored dotted lines reflect confidence intervals.

Non-motor features

MMSE-scores declined faster for PD cases than for controls throughout study follow-up, with significant differences around 5.6 years before diagnosis.(*Figure 3A*) On other cognitive tests, PD cases generally declined more rapidly than controls, and scores on the LDST were earliest to differ with significant worse scores for prediagnostic PD cases from 7.1 years before diagnosis onwards.(*Figure 3B*) Subsequently, PD cases had significantly worse scores on the Stroop tasks 3 (6.2 years; *Figure 3C*), 1 (4.6 years) and 2 (3.8 years), and Word Fluency Test.(3.3 years; *Figure 3D*) Interestingly, PD cases did not more frequently report memory complaints until the last few years before diagnosis.(1.5 years; *Figure 3B*)

Although point estimates for anxiety scores were lower (i.e., worse) for PD cases as early as sixteen years before clinical diagnosis, differences with controls only became significant in the final year before clinical diagnosis.(*Figure 4A*) Interestingly, depressive symptoms were transiently non-significantly more common between among PD cases around fifteen years before PD diagnosis, and became significantly more common among PD cases in the last 2.3 years.(*Figure 4B*) Laxative medication was increasingly used by PD cases throughout study follow-up, and differences with controls became significant 2.4 years before diagnosis.(*Online Supplementary Figure 4C*) During the entire prediagnostic phase, there were no significant differences in systolic blood pressure between PD cases and controls, although systolic blood pressure appeared to rise less steeply over time and even declined somewhat around clinical diagnosis in PD cases.(*Figure 4D*) Similarly, there were no significant differences in diastolic blood pressure between PD cases and controls, but the trajectory was less steep in PD cases.

Figure 3 | Trajectories of non-motor features: cognition.



Y-axis denotes average score for Parkinson's Disease patients and controls separately. Figures 3A: Mini-Mental State Exam (MMSE). 3B: Letter Digit Substitution Test (LDST). 3C: Stroop test task 3. 3D: Word Fluency Test (WFT). PD, Parkinson's Disease. For each group, correspondingly colored dotted lines reflect confidence intervals.

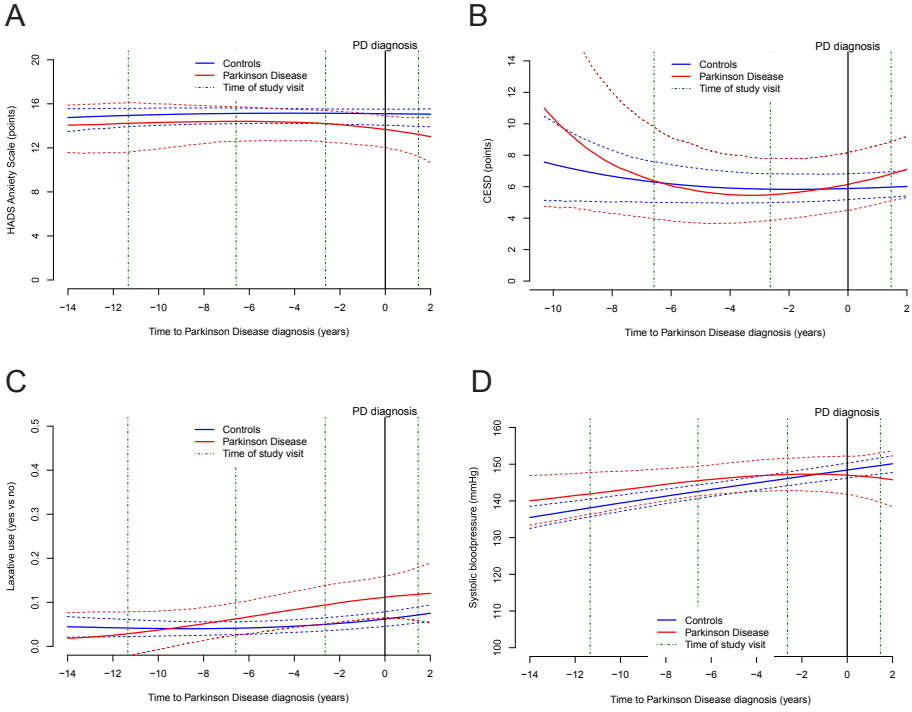
Sensitivity analyses: trajectories of patients using alternative Parkinson's Disease case definitions

During study follow-up, 43 individuals were first diagnosed with dementia and subsequently with parkinsonism during follow-up, and 30 of those did not have a clear secondary cause of parkinsonsim. The mean interval between onset of dementia and onset of parkinsonism in these patients was 1.6 years. Combined prediagnostic trajectories of these patients together with patients who were already classified as PD cases in the main analyses are presented in *Online*

Supplementary Figure 2. Interestingly, differences between patients and controls in IADL (-7.1 years), BADL (-5.6 years) and hypo- or bradykinesia (-8.5 years) occurred at similar timepoints as in the main analyses, whereas the difference in MMSE-scores occurred distinctly earlier (-9.1 years).

In separate sensitivity analyses, only 65 patients met a stricter case definition of PD. Their prediagnostic trajectories of IADL, BADL, hypo- or bradykinesia and MMSE were very similar to the original trajectories.(*Online Supplementary Figure 3*)

Figure 4 | Trajectories of non-motor features: anxiety and depressive symptoms.



Y-axis denotes average score for Parkinson's Disease patients and controls separately. Figures 4A: Hamilton Anxiety and Depression Scale (HADS) anxiety subscale. 3B: Center for Epidemiologic Studies Depression Scale (CES-D). 3C: laxative use. 3D: systolic blood pressure (importantly, these were sitting blood pressure measurements only, not orthostatic blood pressure measurements).Of note, more anxiety corresponds to lower HADS scores. PD, Parkinson's Disease. For each group, correspondingly colored dotted lines reflect confidence intervals.

Chapter 7.1

DISCUSSION

In the years preceding clinical diagnosis, PD patients increasingly experience problems in daily functioning and progressively show both motor and non-motor features. While movement poverty and tremor frequently appear as initial signs of PD, cognitive deficits are also common in early prediagnostic PD patients. These changes are initially reflected by impairments in complex tasks that require both motor and non-motor skills (IADL). The subsequent occurrence of additional features, including motor features such as rigidity, postural imbalance, and postural abnormalities, extends impairment to more physically oriented tasks (BADL).

Before further interpreting the results of this study, several limitations should be noted. First, the presence of motor features was assessed by research nurses, using subjective scores rather than dedicated quantification methods, and since research nurses were trained to classify signs of hypokinesia and signs of bradykinesia at the same time, we could not separately define bradykinesia from these assessments. However, these assessments were not considered for the diagnosis of parkinsonism, and since motor feature assessments were standardized and took place before participants were clinically diagnosed as PD cases or controls, the error that was introduced by their imprecise nature was probably the same in both groups. This suggests that true differences between groups may occur even earlier than we detected. Second, not all PD patients were examined in-person by movement disorder specialists and we lacked histologic confirmation on PD diagnosis, which probably introduced misclassification of PD cases. The detailed in-person and clinical information on the presence and possible causes of parkinsonism throughout the study period make it unlikely that we systematically misclassified one specific group of parkinsonism patients as PD

patients (e.g., drug-induced parkinsonism). Third, we note that while we studied a wide range of features, our approach was not comprehensive; most noteworthy, we did not collect data on REM behavior disorder (RBD) and olfactory function.[28,29] Fourth, we studied a population of individuals aged 55 years and older, and the average age of PD cases was somewhat higher than in general populations that also included younger individuals. Therefore, our findings may not represent the prediagnostic phase of young-onset PD. Fifth, the number of PD cases for whom we had data on the very early prediagnostic phase (i.e., more than 10 years before diagnosis) was relatively low, so some caution is warranted for the interpretation of very early, statistically non-significant differences, most notably for tremor.

To date, two previous reports on long-term trajectories of features in prediagnostic PD patients have been published: a study in RBD patients, who are at extremely high-risk for PD and related α-synucleinopathies,[30] and a registry-based study.[7] Compared to those studies, our approach is novel for several reasons. First, we repeatedly and consistently assessed the presence of a wide range of motor and non-motor features over a time period of up to 23 years, and examinations were identical in individuals who were later diagnosed with PD and in those who were not. During the same period, we continuously followed all participants for the onset of clinical PD and applied consistent, standardized criteria for PD diagnosis. Second, study participants were included from the community irrespective of traits that strongly affect their risk of PD. This is a major difference with studies investigating high-risk populations such as RBD-patients[31] or *GBA*-mutation carriers[32], given that a substantial proportion of prediagnostic PD patients may not have these traits. Therefore, while such studies provide essential insight into prediagnostic changes in subgroups of PD patients,

community-based studies such as the Rotterdam Study harbor a sample of incident patients that is generally representative of the full spectrum of prediagnostic PD in the elderly population. Third, this study identified several novel prediagnostic changes in PD, including cognitive decline and deterioration in instrumental and basic daily functioning.

Importantly, we observed that PD patients already started to decline in daily functioning years before clinical diagnosis. Impairment first became evident in tasks that require a combination of motor and cognitive skills, such as travelling, while problems in more physically oriented tasks increased as additional motor features appeared. Earliest deterioration in daily functioning was paralleled by an increase in movement poverty and slowness, which started to appear more frequently in PD patients compared to controls seven years before diagnosis. Of these, upper limb features such as reduced arm swing and slowed finger tapping preceded changes in gait. These results, as well as our observation that the presence of rigidity typically increased in the last few years before clinical diagnosis, correspond to findings from the RBD-cohort.[30] Furthermore, we observed that tremor presented as an early prediagnostic feature of PD (six years before diagnosis) while postural imbalance increasingly occurred a few years before clinical diagnosis, extending similar findings from the registry-based study.[7] Of motor features, we further note that presence of postural abnormalities and falling increased sharply in the last years before diagnosis.

Strikingly, we also observed declining cognitive function scores in PD patients as early as seven years before clinical diagnosis. Differences with age- and sex-matched controls were generally subtle, however, prediagnostic PD patients in particular declined earlier in processing speed and executive function, whereas memory decline started later. This is in line with the previous observation that

cognitive impairment can be quite advanced in clinical PD patients with relatively high MMSE-scores.[33] Of note, in our study, the follow-up for LDST, Stroop test and WFT was shorter than for MMSE, which may have prevented us from detecting statistically significant differences between PD patients and controls at an even earlier moment for these tests. Furthermore, the tests that we used relatively underrepresented specific cognitive dysfunction of PD, such as attentional-executive dysfunction and impaired visuoperceptual skills. Specific tests designed for cognitive function in PD patients may have detected differences between PD patients and controls at an even earlier moment, possibly also showing greater amplitude of differences.

Interestingly, we observed that as impairments in daily functioning rose, prediagnostic PD patients increasingly reported anxiety symptoms. Similarly, PD patients increasingly reported depressive symptoms, but differences with controls only became significant around diagnosis. Of other non-motor features, laxative use among PD patients increased years before clinical diagnosis, which is similar to findings on constipation from another population-based study.[34] However, differences between PD patients and controls were only significant for a small part of the prediagnostic period. This may be caused by our analysis method of treating laxative use as a proxy for constipation, which probably led to an underestimate of its true prevalence. Furthermore, in contrast to previous studies which identified hypotension as a risk factor for PD,[35] we did not observe lower blood pressures for PD patients across prediagnostic trajectories. Importantly, these were sitting blood pressure measurements only, not orthostatic blood pressure measurements. Repeated measures on orthostatic hypotension may be more sensitive to detect early autonomic function deterioration in prediagnostic PD patients.

Decline in daily functioning is increasingly recognized as an important determinant of quality of life in PD patients,[36] and several trials have shown that self-perceived performance in daily activities by PD patients can be improved by non-invasive interventions.[37,38] At the same time, symptomatic treatment modalities for various motor and non-motor features of early clinical PD are advancing.[39-41] Our observation that decline in daily functioning starts years before clinical parkinsonism may open the door to even earlier symptomatic intervention trials (i.e., in prodromal PD patients), and our data on the occurrence of prediagnostic features (most notably: objective cognitive decline) may aid in the identification of individuals who would benefit most from such early symptomatic treatment. However, we note that while our approach (nested case-control analyses using repeated assessments in a general population) is very powerful to detect early differences between prediagnostic PD patients and controls, the relative risk estimates presented graphically cannot be directly interpreted as prospective predictive estimates for incident PD, such as the risk estimates in the recent MDS criteria for prodromal PD.[42] In a previous paper, we investigated whether a single, rapid assessment of non-motor features, both separately and combined, could identify individuals at high risk of PD.[12] That method is less powerful than the method used here, but given its simple and rapid nature probably more closely mirrors potential future population-wide screening for PD. Therefore, while we believe that assessments of IADL, BADL and objective cognitive functioning may be useful additions to the MDS criteria for prodromal PD, their prospective predictive value is still to be formally examined.

Furthermore, while our observation of autonomic and limbic features in prediagnostic PD is in line with Braak's theory,[43] our observation of movement poverty and slowness as well as cognitive decline years before clinical diagnosis

are not. Taken together, these findings support the notion that pathological processes of PD may already be too advanced in newly diagnosed patients for putative protective interventions to have substantial effects.[3] Data from this study may provide context for selection of individuals for neuroprotective trials that target PD patients before a clinical parkinsonism diagnosis is possible.

In conclusion, prediagnostic PD patients often first experience problems in instrumental daily activities, in parallel with the occurrence of early motor and cognitive deficits. Once additional prediagnostic features arise, decline in daily functioning accelerates and extends to problems in more physically oriented tasks.

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Chapter 7.2

Cognitive functioning

ABSTRACT

Cognitive dysfunction is a common feature among patients with parkinsonism, including Parkinson's Disease (PD). However, there is a scarcity of data on cognitive functioning before parkinsonism diagnosis, a stage at which patients may still respond to putative disease-modifying interventions. We assessed whether poor cognitive functioning is associated with an increased risk of parkinsonism. Between 2002 and 2008, we assessed baseline cognitive functioning in 7,386 participants of the Rotterdam Study who were free of parkinsonism and dementia. We used four tests (Stroop, Letter-Digit Substitution, Verbal Fluency, Word Learning) and derived a global cognition score from principal component analysis. Subsequently, we followed participants until January 1, 2015 for the onset of parkinsonism through serial in-person examinations and complete access to medical records. Parkinsonism was defined as at least one of: 1) the presence of hypo- or bradykinesia plus at least one other cardinal sign; 2) clinical diagnosis by a neurologist or geriatrician. Probable PD included patients with dementia diagnosis before parkinsonism diagnosis. During follow-up (median 8.3 years), 79 individuals were diagnosed with incident parkinsonism, 57 (72%) with probable PD. Among incident parkinsonism patients, 24 also developed dementia (10 before and 14 after parkinsonism onset). Poor global cognition at baseline was associated with a higher hazard of incident parkinsonism ([age-, sex- and subcohort-adjusted] hazard ratio [per standard deviation decrease in global cognition]=1.79, 95% confidence interval [1.37; 2.33]). The association remained robust even beyond the first eight years (hazard ratio=1.59 [1.01; 2.59]) and after removing individuals with dementia onset before parkinsonism (hazard ratio=1.72 [1.28; 2.27]). Poor global cognition at baseline was also associated with incident probable PD (hazard ratio=1.52 [1.10; 2.08]).

Letter-Digit Substitution (hazard ratio=1.56 [1.20; 2.04]), Verbal Fluency (hazard ratio=1.54 [1.25; 1.92]), and inverted interference-task Stroop (hazard ratio=1.35 [1.10; 1.69]) scores were each strongly associated with incident parkinsonism, whereas the association of delayed-task Word Learning scores was distinctly weaker (hazard ratio=1.16 [0.91; 1.49]). In conclusion, poor cognitive functioning is associated with an increased risk of incident parkinsonism, including probable PD. Importantly, cognitive functioning predicts parkinsonism even over long intervals, and extends beyond patients with onset of parkinsonism after dementia. Our findings suggest that cognitive dysfunction can be considered a sign of prodromal PD.

BACKGROUND

Poor cognitive functioning is common among patients with parkinsonism, and a substantial subgroup of patients already has cognitive dysfunction at the time of parkinsonism diagnosis. Most notably, between 15%-43% of newly diagnosed Parkinson's Disease (PD) patients are cognitively impaired,[1,2] and cognitive dysfunction at diagnosis is a risk factor for subsequent dementia in PD patients.[3] In patients with dementia with Lewy Bodies the dementia diagnosis typically precedes parkinsonism onset. In addition, poor cognitive functioning is common among patients with multiple system atrophy, progressive supranuclear palsy and vascular parkinsonism.[4-6]

Interestingly, cognitive functioning has also been reported worse in individuals who are free of parkinsonism but have impaired olfaction and reduced dopamine transporter binding, both of which are strong proxies of PD.[7] Recently, in a case-control setting we showed that subtle decline in executive cognitive functions may precede diagnosis in PD patients, in parallel with subtle motor signs.[8] In addition, we showed that poor manual dexterity is associated with an increased risk of incident parkinsonism and PD, even when measured years before diagnosis.[9]

However, it is unclear whether a single global assessment of cognitive functioning is associated with an increased risk of parkinsonism over longer intervals, to what extent the different cognitive domains predict parkinsonism, and how poor cognitive functioning combines with subtle motor signs in prediagnostic parkinsonism patients. Such insight is critical, because it may further unravel the prodromal phase of common parkinsonism diseases, in particular prodromal PD,

a phase in which patients may still respond to putative disease-modifying interventions.

We hypothesized that poor cognitive functioning is associated with an increased risk of parkinsonism and tested this hypothesis in a prospective, population-based study. Furthermore, we hypothesized that such an association would be especially driven by performance on executive functions, and that a combination of poor cognitive functioning and subtle motor signs would be a strong predictor of incident parkinsonism.

METHODS

Study population

The study was embedded in the Rotterdam Study (RS), a large, prospective, population-based study in the Netherlands.[10,11] In 1990, inhabitants of the well-defined Ommoord district in the city of Rotterdam who were aged 55 years and older were invited to participate, and 7,983 individuals agreed (first subcohort). In 2000, all inhabitants who had become 55 years of age and older, or moved into the study district since the start of the study were invited to be included in the Rotterdam Study, and 3,011 agreed (second subcohort). The cohort was further extended in 2006 (third subcohort; age range 45 years and older) to a total of 14,926 participants (overall response 72.0%). By 2015, the first subcohort had a total of six visits (mean interval between visits: eight years), whereas the second subcohort had three visits, and the third subcohort had two.

Although cognitive screening tests had been employed since 1990, a more comprehensive cognitive test battery was introduced during center visit rounds in

2002-2004 (first subcohort), 2004-2006 (second subcohort) and 2006-2008 (third subcohort). Of the original 14,926 participants, 8,414 were still alive at the time of and participated in this broader assessment, and we will refer to this assessment as "baseline". Participants were extensively screened for parkinsonism and dementia,[12,13] and we excluded individuals who had parkinsonism or dementia at baseline as well as individuals who had missing data on more than one cognitive functioning task (n=1,028). We followed the remaining 7,386 participants until the first of: onset of parkinsonism, onset of dementia, January 1, 2015, or death. Study follow-up for incident parkinsonism was virtually complete (98%).[14]

Assessment of cognitive functioning and manual dexterity

We previously published a detailed description of our assessment methods of objective cognitive functioning as well as age- and sex-specific normative values.[15] In short, we used the Stroop color word test (comprising three tasks),[16] Letter-Digit Substitution Test (LDST),[17] Verbal Fluency Test,[18] and 15-Word List Learning Test (WLT).[19] In *Table 2*, the domain that is primarily represented by each test is listed. Of these domains, executive abilities, information processing speed, attention and semantic fluency are commonly impaired in clinical PD patients, including in PD-MCI patients.[20-22] Furthermore, impairment in semantic fluency is associated with subsequent risk of dementia in PD patients.[23]

We used the Purdue Pegboard Test to assess manual dexterity.[24] In this test, participants are tasked to place as many cylindrical metal pegs into one of 25 holes in a pegboard as possible in 30 seconds. The test is performed thrice, using left hand, right hand, then both hands simultaneously. The average Purdue score is the sum of each trial divided by 3.

Table 2 | Description of cognitive tests.

Test	Latent domain	Task description
Global cognition	*(summary measure of cognitive tests)*	
Letter-Digit Substitution*	Information processing speed, executive function	Make as many letter-digit combinations as possible in 60 seconds (following an example that shows correct combinations)
Stroop task 1	Speed of reading	Name the printed words on a colored card with 40 items; outcome is the time to complete the task
Stroop task 2	Speed of color naming	Name the printed colors the same card as in Stroop task 1; outcome is the time to complete the task
Stroop task 3*	Interference of automated processing and attention	Name the color in which each color-name is printed on the same card as in Stroop task 1; outcome is the time to complete the task.
Verbal Fluency*	Semantic fluency	Name as many animals as possible within 60 seconds
World learning immediate task	Verbal learning	Memorize a sequence of 15 words (which was presented 3x); subsequently immediately recall as many of these words as possible
World learning recognition task	Recognition from verbal memory	Memorize the 15 words presented during the immediate recall mixed with 30 new words; subsequently recognize the words from the immediate recall sequence; outcome is the number of correctly recognized words
World learning delayed task*	Retrieval of verbal memory	Memorize a sequence of 15 words (3x), and recall after 15 minutes as many of these words as possible; outcome is the number of correctly remembered words

Chapter 7.2

Assessment of parkinsonism

A detailed description of assessment methods is provided in **Chapter 4.1**. In short, we used four overlapping modalities to screen for potential parkinsonism: in-person examinations (on average every 4 years), in-person interviews, use of antiparkinson medication, and clinical monitoring alerts.

Of all individuals who screened positive in any of these methods, complete medical records (including letters from medical records of specialists and general practitioners) were studied and case reports were drawn up covering all potentially relevant information to establish presence and cause of parkinsonism. These case reports were evaluated by a panel led by an experienced neurologist.

Parkinsonism was defined as at least one of: 1) the presence of hypo- or bradykinesia in combination with at least one other cardinal sign (resting tremor, rigidity or postural imbalance) as observed by any physician; 2) a clinical diagnosis of parkinsonism by a neurologist or geriatrician.

Assessment of probable Parkinson's Disease

As described in detail in in **Chapter 4.1**, we previously defined PD as parkinsonism not associated with a secondary cause and with at least one of: 1) a clinical PD diagnosis by a neurologist or geriatrician; 2) positive response to antiparkinson medication. It is noteworthy, however, that during the study period (2002-2015) dementia at parkinsonism diagnosis was considered a secondary cause of parkinsonism and therefore used as an exclusion criterion for PD. As a consequence, there were parkinsonism patients with a clinical presentation consistent with PD -and without another clear secondary cause of parkinsonism-

whom were not originally classified as PD because their parkinsonism was preceded by a dementia diagnosis (n=9). In the recent MDS criteria of PD,[25] prior dementia is no longer an exclusion criterion for PD, suggesting that these patients would now likely receive a clinical PD diagnosis. Therefore, in this study we classified these 9 patients together with the patients who had already received a clinical PD diagnosis (n=48) as 'probable PD' patients.

Assessment of dementia

A detailed description of assessment methods has previously been published.[26] In short, participants were screened for dementia at baseline and follow-up examinations using a three-step protocol,[13] Individuals with a positive screen on either Mental State Examination (MMSE) [27] or the Geriatric Mental Schedule (GMS) organic level [28] were subjected to the Cambridge Examination for Mental Disorders of the Elderly.[29] Additional information was obtained from routinely performed in-person neuropsychological examination, and the total cohort was continuously monitored for dementia through computerised linkage of medical records from general practitioners and the regional institute for outpatient mental healthcare with the study database. Available neuroimaging data were used when required for establishing a diagnosis. Basic ADL and instrumental ADL were routinely assessed at every center visit, using the Stanford Health Assessment Questionnaire and Lawton Instrumental, respectively. Additional interview data on ADL impairment during between-visit intervals was obtained from medical records. For all suspected cases of dementia, a consensus panel led by a consultant neurologist, decided on the final diagnosis in accordance with standard criteria for dementia (DSM-III-R).

Definition of cognitive dysfunction and subtle motor features

Since this is a population-based study, we used internal age- and sex-specific norms in the full cohort to determine the presence of cognitive dysfunction. Age- and sex-specific norms of cognitive test scores in the Rotterdam Study have previously been published. [15] Cognitive dysfunction was defined as age- and sex-adjusted global cognition z-score <-1. Subtle motor features were defined as any parkinsonian sign during screening or <-1 Purdue Pegboard z-score compared to age- and sex specific internal norms.

Statistical analysis

Cognitive test scores were visually inspected for skewedness; Stroop test scores were right-skewed and subsequently log-transformed to obtain a roughly normal distribution. Furthermore, to facilitate comparison of effect sizes with other tests, all test scores were standardized (i.e., were converted to z-scores) and Stroop scores were multiplied by -1.

Different cohort studies often use different batteries of cognitive tests, making it difficult to compare findings of separate test scores across studies. However, g-factor scores of cognitive function tests (global cognition) derived from different cognitive test batteries are very similar,[30,31] and typically explain approximately 50% of all variance in the cognitive tests.[32] The g-factor is identified as the first unrotated component of a principal component analysis that incorporates tasks from all available cognitive function tests. Although Stroop and WLT comprised several tasks, we only used data from the most complicated task of each (i.e., the

interference task for Stroop and the 15-minute delayed recall task for WLT) in calculating the g-factor to prevent highly correlated tasks distorting factor loadings in the principal component analysis. Contrary to our previous work,[15] we did not include the Purdue Pegboard test in the g-factor, since it is strongly influenced by motor function and was previously already shown to predict incident parkinsonism in this population.[9]

We studied the association of cognitive function test scores with incident parkinsonism using Cox proportional hazards models, with adjustment for age, sex and study subcohort. In sensitivity analyses, we repeated the main analysis after: 1) exclusion of the first five years of follow-up; 2) exclusion of individuals with subtle motor features at baseline (any parkinsonian sign during screening or $\leq$-1 Purdue Pegboard z-score compared to internal age- and sex specific norms); 3) censoring (i.e. treating these individuals as not having incident parkinsonism) patients with incident dementia preceding parkinsonism (censored at time of dementia diagnosis); 4) censoring patients who were not diagnosed by a neurologist or geriatrician; 5) restriction to probable PD patients. Furthermore, in other sensitivity analyses, we explored whether an association between cognitive performance and incident parkinsonism in mid- or late-life may be reflective of early life cognitive performance by additionally adjusting the main model for level of education, and separately assessed effect modification by age, gender or study subcohort by introducing one-by-one interaction terms with global cognition.

To enable translation of our findings to clinical practice, we also present likelihood ratios (LRs) for the baseline presence of isolated or combined cognitive dysfunction and subtle motor features (any parkinsonian sign or low Purdue Pegboard score) for incident parkinsonism during follow-up. We used two different thresholds to define cognitive dysfunction and low Purdue Pegboard

score (≤-1 and ≤-2 z-score compared to internal age-&sex-specific norms). Furthermore, we consecutively subtracted 1 year of follow-up in a step-wise fashion to explore whether the LRs of combined cognitive dysfunction and subtle motor features (at baseline), for incident parkinsonism during follow-up, varied by duration of follow-up. Of note, since the Rotterdam Study is a population-based study, we used internal age- and sex-specific norms in the full cohort to determine the presence of cognitive dysfunction. Age- and sex-specific norms of cognitive test scores in the Rotterdam Study have previously been published [15]

The MMSE was not included in the main analyses because it is less sensitive to cognitive performance than the separate cognitive function tests, in large part due to ceiling effects. Still, since the MMSE is easily applied clinically, we also studied the association of total MMSE score with incident all-cause parkinsonism and incident probable PD, and repeated the main analyses of Global cognition after incorporation of the total MMSE score. As visuospatial impairment is a common sign in clinical PD, we separately repeated these sensitivity analyses using the copy item of the MMSE only.

155 study participants (2%) did not complete one cognitive functioning task; to avoid selective loss of data, we performed multiple imputation using the mean of five imputations, based on educational attainment, other test scores, incident dementia status, and incident parkinsonism status. The distribution of test scores before and after imputation was similar. Data were handled and analyzed with the IBM SPSS Statistics version 23.0.0.0 (IBM Corp., Somers, NY) and R version 3.2.4.

RESULTS

Baseline characteristics of the study population are shown in *Table 1*. Individuals with at least 1 parkinsonian sign had lower Purdue Pegboard scores than other individuals (age-and sex-adjusted difference in Purdue z-score 0.25 [p<0.001]). In individuals without any parkinsonian sign, cognitive dysfunction was associated with lower scores on the Purdue Pegboard (age- and sex-adjusted difference in Purdue z-score: 0.48 [p<0.001]).

During follow-up, 7,386 participants amassed 61,660 person-years (median 8.3 years), 79 (1.1%) participants were diagnosed with incident parkinsonism. Of those with incident parkinsonism, 24 (30%) were also diagnosed with incident dementia (10 before and 14 after onset of parkinsonism), and 446 (6%) individuals who remained free of incident parkinsonism were diagnosed with incident dementia. The median interval between baseline cognitive assessment and detection of incident parkinsonism was 5.6 years. 54 (68%) patients were diagnosed with parkinsonism without a structured physical examination by a research physician, whereas 13 (16%) patients were diagnosed without a diagnostic examination by a neurologist or geriatrician. Overall, 8 (10%) patients had neither; these patients were diagnosed by a general practitioner or nursing home physician. Of parkinsonism patients, 57 (72%) were diagnosed with probable PD. A complete overview of clinical parkinsonism diagnoses is presented in *Online Supplementary Table 1*.

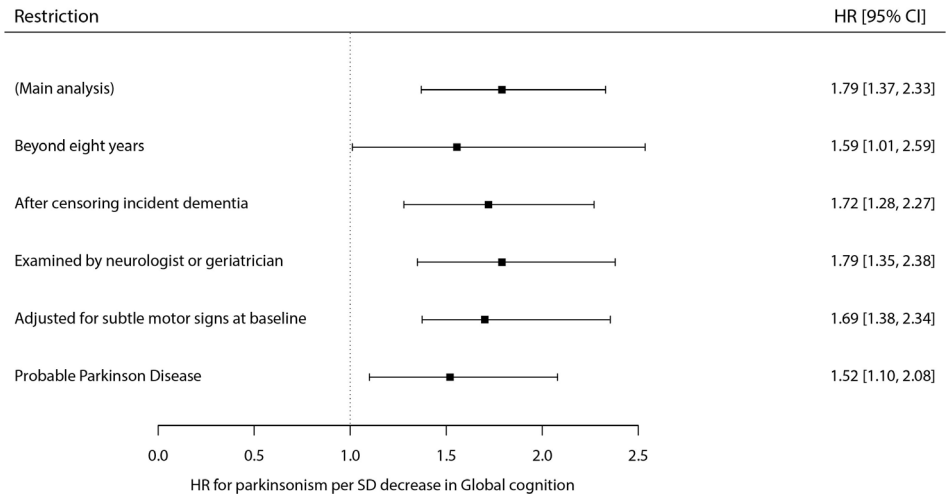
Table 1 | Study population characteristics.

Characteristic	Population at risk [n=7,386]
Age, years	65.3 [10.2]
Female sex	4236 [57.4]
Education	
Primary education only	760 [10.4]
Lower/intermediate general education or lower vocational education	2994 [40.8]
Intermediate vocational education or higher general education	2165 [29.5]
Higher vocational education or university	1419 [19.3]
≥ 1 Parkinsonian sign at baseline	936 [12.7]
Purdue Pegboard, mean points per task	11.8 [1.8]
Letter-Digit Substitution*, number of correct answers [possible scoring interval 0-135]	29.4 [7.3]
Stroop task 1, time to complete, seconds	17.5 [3.9]
Stroop task 2, time to complete, seconds	23.5 [4.9]
Stroop task 3*, time to complete, seconds	50.5 [19.3]
Verbal Fluency*, number of correct answers	22.3 [5.8]
World learning immediate task, mean number of words per trial [possible scoring interval 0-15]	7.4 [2.1]
World learning recognition task, number of words [possible scoring interval 0-15]	13.2 [2.1]
World learning delayed task*, number of words [possible scoring interval 0-15]	7.3 [2.9]

*For continuous characteristics, mean [standard deviation] is presented; for categorical characteristics, number [percentage] is presented. Parkinsonian signs: hypo- and bradykinesia, tremor, cogwheel rigidity, and postural instability. *Used in calculation of Global cognition.*

Poor global cognition at baseline was associated with a higher risk of incident parkinsonism (hazard ratio per standard deviation decrease HR=1.79, 95% confidence interval [1.37; 2.33]). Interaction terms of global cognition with age (p=0.27), sex (p=0.54), and study subcohort (p>0.25 for each subcohort) were not statistically significant. The association between poor cognitive functioning and incident parkinsonism remained robust beyond the first eight years, after censoring individuals with incident dementia, and after restricting the analysis to incident parkinsonism cases who were examined by a neurologist or geriatrician.(*Figure 1*)

Figure 1 | The association between global cognition and incident parkinsonism: sensitivity analyses.



HR, hazard ratio for incident parkinsonism per standard deviation decrease in cognitive functioning score. SD, standard deviation. CI, confidence interval. Analyses are adjusted for age, sex, and study subcohort. 3963 individuals were included in the analysis for incident parkinsonism beyond 8 years, including 22 individuals who were diagnosed with incident parkinsonism after the first 8 years. 17 (77%) of these patients had an interim visit between years 5 and 8.

Also, additional adjustment for educational attainment did not affect the association between poor cognitive functioning and incident parkinsonism (HR=1.81 [1.37; 2.38]). The association only slightly weakened after additional adjustment for subtle motor signs (HR=1.69 [1.38; 2.34]).(*Figure 1*) The association attenuated somewhat and was no longer statistically significant after exclusion of individuals with subtle motor signs at baseline (HR=1.33 [0.92; 1.92]). Poor cognitive functioning was also associated with probable PD (HR=1.52 [1.10; 2.08]) and with a joint endpoint of probable PD or Dementia with Lewy Bodies (HR=1.59 [1.17; 2.17]). With respect to the separate cognitive functioning tests, LDST, Stroop tasks 1 and 3, verbal fluency, and immediate WLT scores were significantly associated with all-cause parkinsonism, each with a hazard ratio exceeding 1.3 per standard deviation decrease (*Table 3*). For probable PD, effect estimates were generally direction-consistent but somewhat attenuated and not statistically significant, with the exception of verbal fluency.(*Table 3*)

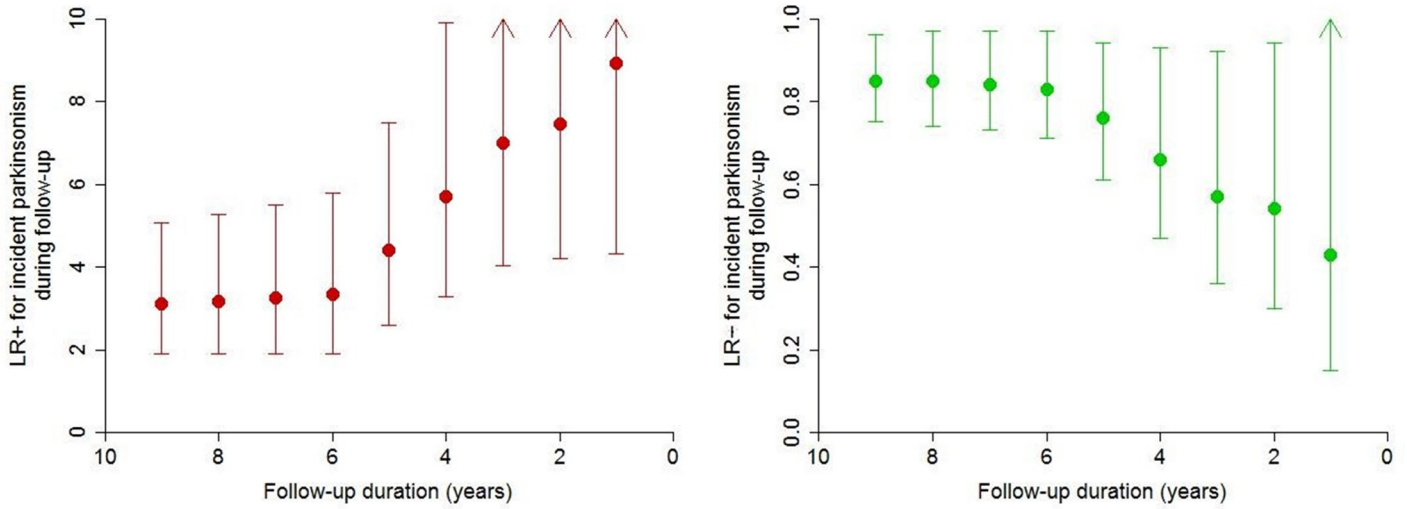
Almost half (49.4%) of participants who were diagnosed with incident parkinsonism during follow-up already had subtle motor features at baseline, cognitive dysfunction at baseline, or both. As shown in *Table 4*, individuals with cognitive dysfunction at baseline had an almost doubled risk of incident parkinsonism compared to individuals with normal cognitive functioning. Individuals who had both cognitive dysfunction and subtle motor signs had at baseline a 3.3-fold increased risk of incident parkinsonism. This translates to a moderately increased posttest probability of developing incident parkinsonism for individuals who had both features at baseline (positive likelihood ratio LR+=2.66), and the LR+ increased further after follow-up was restricted to shorter intervals.(*Figure 2*)

Table 3 | Poor cognitive functioning and the risk of all-cause parkinsonism and probable Parkinson's Disease.

Test		All-cause Parkinsonism	Probable PD		
	Mean [SD] No incident PS	Mean [SD] Incident PS	HR [95% CI]	Mean [SD] Incident probable PD	HR [95% CI]
Global cognition	*0.01 [1.00]*	*-0.71 [1.00]*	*1.79 [1.39; 2.33]*	*-0.59 [1.08]*	*1.52 [1.11; 2.08]*
Letter-Digit Substitution*	0.00 [1.00]	-0.62 [0.97]	1.59 [1.22; 2.04]	-0.48 [1.04]	1.32 [0.96; 1.79]
Stroop task 1	0.00 [1.00]	-0.39 [1.25]	1.61 [1.23; 2.08]	-0.24 [1.35]	1.35 [0.99; 1.82]
Stroop task 2	0.01 [1.00]	-0.50 [1.24]	1.18 [0.94; 1.47]	-0.40 [1.30]	1.02 [0.78; 1.33]
Stroop task 3*	0.01 [1.00]	-0.70 [1.09]	1.37 [1.10; 1.69]	-0.70 [1.17]	1.23 [0.95; 1.61]
Verbal Fluency*	0.00 [1.00]	-0.53 [1.00]	1.56 [1.25; 1.96]	-0.40 [0.98]	1.56 [1.20; 2.00]
World learning immediate task	0.00 [1.00]	-0.44 [1.01]	1.32 [1.02; 1.69]	-0.37 [1.01]	1.19 [0.88; 1.59]
World learning recognition task	0.00 [0.99]	-0.34 [1.47]	1.19 [0.99; 1.43]	-0.38 [1.46]	1.22 [0.99; 1.49]
World learning delayed task*	0.00 [1.00]	-0.34 [1.00]	1.18 [0.92; 1.52]	-0.23 [1.00]	1.02 [0.77; 1.37]

*PS, parkinsonism. PD, Parkinson Disease.Stroop scores were log-transformed and multiplied by -1; all scores were standardized as z-scores (i.e. 0=average for the group). HR, hazard ratio for incident parkinsonism per standard deviation decrease in cognitive functioning score. Analyses are adjusted for age, sex and study subcohort. *Used in calculation of Global cognition. Latent domain indicates the domain that a test primarily represents.*

Figure 2 | Combined cognitive dysfunction and subtle motor feature(s) at baseline: likelihood ratios for incident parkinsonism, by decreasing follow-up duration.



LR+, positive likelihood ratio. LR-, negative likelihood ratio.

Positive and negative likelihood ratio of having both cognitive dysfunction and >1 subtle motor feature (at baseline), for incident parkinsonism during follow-up. Reference: isolated cognitive dysfunction, isolated subtle motor signs, or normal cognitive and motor functioning. Cognitive dysfunction, <-1 Global cognition z-score compared to age-&sex-adjusted means. Subtle motor feature, any parkinsonian sign or <-1 standard deviation manual dexterity score compared to age-&sex-adjusted means.

The LR+ of cognitive dysfunction and subtle motor features increased from 2.66 [1.64; 4.32] for incident parkinsonism during the entire follow-up to 8.91 [4.33; 18.32] for incident parkinsonism during the next year, while the LR- decreased from 0.88 [0.80; 0.98] to 0.43 [0.15; 1.25]. Cognitive dysfunction, <-1 Global cognition z-score compared to age-&sex-adjusted means. Subtle motor feature, any parkinsonian sign or <-1 standard deviation manual dexterity score compared to age-&sex-adjusted means. The LR+ of cognitive dysfunction and subtle motor features increased from 2.66 [1.64; 4.32] for incident parkinsonism during the entire follow-up to 8.91 [4.33; 18.32] for incident parkinsonism during the next year, while the LR- decreased from 0.88 [0.80; 0.98] to 0.43 [0.15; 1.25].

Table 4 | Practical estimates for prediction of parkinsonism: cognitive dysfunction, subtle motor features, or both.

Cut-off	N no incident PS [%]	N incident PS [%]	HR [95% CI]	LR+	LR-	Cut-off
-1 SD	Cognitive dysfunction*	1159 [15.9]	22 [27.8]	1.97 [1.25; 3.11]	1.76 [1.23; 2.51]	0.86 [0.75; 0.98]
	Any subtle motor features**	1825 [25.0]	31 [39.2]	2.33 [1.42; 3.82]	1.57 [1.19; 2.07]	0.81 [0.68; 0.97]
	Cognitive dysfunction + any subtle motor feature***	486 [6.7]	14 [17.7]	3.39 [1.89; 6.05]	2.66 [1.64; 4.32]	0.88 [0.80; 0.98]
-2 SD	Cognitive dysfunction*	211 [2.9]	7 [8.9]	2.18 [1.31; 3.62]	3.07 [1.49; 6.30]	0.94 [0.88; 1.01]
	Any subtle motor features**	1079 [14.8]	22 [27.8]	4.45 [2.04; 9.72]	1.89 [1.32; 2.70]	0.85 [0.74; 0.97]
	Cognitive dysfunction + any subtle motor feature***	64 [0.9]	4 [5.1]	9.52 [3.46; 26.20]	5.78 [2.16; 15.49]	0.96 [0.91; 1.01]

HR, hazard ratio for incident parkinsonism. 95% CI, 95% confidence interval. Analyses are adjusted for age, sex and subcohort. Cognitive dysfunction, <-1 or <-2 Global cognition z-score compared to age-&sex-adjusted means. Subtle motor feature, any parkinsonian sign or <-1 or <-2 standard deviation manual dexterity score compared to age-&sex-adjusted means. Cut-off, threshold for cognitive dysfunction or low manual dexterity score.
**Reference: normal cognitive functioning, irrespective of the presence of motor features. ** Reference: no subtle motor features, irrespective of cognitive functioning. ***Reference: isolated cognitive dysfunction, isolated subtle motor signs, or normal cognitive and motor functioning. After restriction to incident probable Parkinson's Disease patients, the LR+ of combined cognitive dysfunction and subtle motor signs only mildly attenuated (LR+=2.36 [1.29; 4.32] for <-1 z-score threshold; LR+=8.03 [3.03; 21.33] for <-2 z-score threshold).*

When a stricter threshold for cognitive dysfunction and low manual dexterity was used (≤-2 z-score), the LR+ of combined cognitive dysfunction and subtle motor features at baseline also increased.*(Table 4)*

Of 1,181 individuals with cognitive dysfunction at baseline, 486 also had subtle motor signs at baseline (41%), including the majority (14/22 [64%]) of those who were diagnosed with both incident parkinsonism and incident dementia.(*Online Supplementary Table 2*) In individuals who were diagnosed with incident dementia and also with incident parkinsonism, baseline cognitive dysfunction was not associated with incident dementia (HR=1.10 [0.43; 2.80]).

The MMSE was not included in the main analyses because it is less sensitive to subtle cognitive deficits than the other cognitive functioning tests. Still, lower total MMSE score was associated with higher risk of incident all-cause parkinsonism and non-significantly with incident probable PD.(*Online Supplementary Table 3*) The pentagon copy item of the MMSE was also associated with an increased risk of incident all-cause parkinsonism and also non-significantly with incident probable PD. Inclusion of the pentagon copy in global cognition did not substantially affect its association with incident parkinsonism.(*Online Supplementary Table 3*)

DISCUSSION

In this prospective, population-based cohort, poor cognitive functioning was associated with an increased risk of parkinsonism. This association was present even after restricting analysis to incident parkinsonism patients without dementia. It is also present for diverse domains of cognition, including executive, attention,

cognitive speed, and memory domains. Our findings suggest that cognitive dysfunction can be considered a sign of prodromal PD.

Before further interpreting these findings, several methodological considerations should be noted. First, it is possible that some misclassification of the diagnosis of parkinsonism occurred. However, after restricting our analyses to patients who were examined by neurologists or geriatricians, we observed a similar overall association. Furthermore, we classified patients who had a clinical presentation consistent with PD as probable PD patients irrespective of whether and when they also developed dementia. This was done to be consistent with the 2015 MDS clinical diagnostic criteria for PD,[25] in which PD and DLB are also no longer considered mutually exclusive diagnoses. We note that it is likely that many of these patients would also have met criteria for DLB once additional features developed. However, the removal of early dementia as an exclusion criterion for PD diagnosis remains a highly debated issue, and there are opposing viewpoints.[33,34] Strengths of our study include its population-based design, which ensures a representative sample of unselected incident parkinsonism patients, active screening for parkinsonism and dementia at baseline, complete access to medical files of study participants (including specialist letters), and the extensive (median 8 years) follow-up for incident parkinsonism. This study extends findings from our recently published nested case-control study,[8] in which we showed that poor executive functioning and subtle motor signs are increasingly more common among prodromal PD patients. While our previous publication documented the temporal relationship, it could not be used to estimate risk with a single measure, which is highly needed to further refine diagnostic criteria for prodromal PD.[35] In this project, we used a prospective design based on a single global cognitive functioning measure, which allowed us to assess how common poor cognitive

functioning is in prodromal parkinsonism patients, both in isolation as well as in combination with subtle motor features.

We observed that poor cognitive functioning is prospectively associated with parkinsonism, also when analyses were restricted to patients with probable PD. Our data provide important insight into the association of cognitive functioning with incident parkinsonism in the general population, especially given the fact that only two population-based studies have previously published data on this topic.[36,37] In the Honolulu Asia Aging study of Japanese-American men, an increased risk of PD was seen across quartiles of worse executive function as well as an increased risk with slow reaction time (the full study results have not yet been published).[36] In the Religious Orders Study and Memory and Aging Project, associations between several cognitive functioning tests and incident parkinsonism (without diagnosis of PD vs. atypical parkinsonism) were reported in an elderly population with 5-year follow-up.[37] Separately, in a cross-sectional study of parkinsonism-free individuals, participants with strong PD proxies (hyposmia and dopamine transporter binding reduction) had worse global cognition than other participants, and particularly performed worse on tasks relating to fluency, task switching, attention and working memory.[7] The observations in these three studies are consistent with our findings: we observed strong associations between incident parkinsonism and test scores relating to fluency, and moderate associations with processing speed, reading speed, interference of automated processing and attention, and recognition from verbal memory. These results are also in line with the observation that non-amnestic impairment is more common than amnestic impairment in clinical PD patients with MCI.

Associations of separate cognitive functioning tests with probable PD were generally direction-consistent. However, with the exception of verbal fluency and the word learning recognition task, hazard ratios were lower for probable PD than for all-cause parkinsonism. Also, most associations of separate cognitive functioning tests with probable PD were statistically (narrowly) non-significant; this may be due to the relatively small number of incident probable PD cases and the fact that the tests used were not designed specifically to detect cognitive dysfunction in PD.[38]

We consider three explanations for an observed link between poor cognitive functioning and incident parkinsonism. The first would be that parkinsonism only precedes cognitive dysfunction. This is a highly unlikely explanation for our results. We actively screened for parkinsonism and dementia at baseline, and excluded individuals with baseline parkinsonism (and dementia). Furthermore, the association remained robust beyond the first eight years, making it unlikely that our findings were driven by a delay in parkinsonism diagnosis. The second would be that individuals destined to develop parkinsonism in mid- or late-life never attain a high level of cognitive functioning in early life. However, adjustment for education did not affect the association between cognitive functioning and incident parkinsonism in our population. Still, since educational attainment is only a proxy of early life cognitive functioning, we cannot completely exclude this explanation. Third, low baseline cognitive scores may indicate ongoing cognitive decline in prediagnostic parkinsonism patients, most of whom have PD. Consistent with this explanation is the high proportion (30%) of incident parkinsonism patients who were also diagnosed with incident dementia during follow-up, either before or after parkinsonism diagnosis. Interestingly, almost half of patients who were diagnosed with incident parkinsonism during follow-up

already had some cognitive dysfunction, subtle motor features at baseline, or both. These findings suggest that both motor and cognitive decline are common in prodromal PD patients, and that their sequence of occurrence is variable. Future observational studies will unravel the underlying pathologies that drive the variable sequence of motor and cognitive decline in prodromal PD patients.

In the MDS criteria for prodromal PD, cognitive loss was not included as a variable, because of the absence of prospective evidence of predictive value.[35] Our results, as well as those of other published studies suggest that cognitive dysfunction now warrants inclusion as a prodromal marker. Of note, while subtle motor abnormalities and cognitive dysfunction often occur in the same individuals, their predictive utility for incident parkinsonism only slightly overlaps. In this study, having a global cognitive score 1 SD below the mean would be associated with a positive likelihood ratio (LR+) of 1.97 for parkinsonism (whereas normal cognition would translate to a LR- of 0.86), and combined cognitive dysfunction and subtle motor signs was an even stronger predictor for parkinsonism. The predictive value further increased for more stringent thresholds of cognitive and subtle motor dysfunction, and also for shorter prediction intervals. The correlation between subtle motor features and cognitive dysfunction will likely also be taken into account in risk estimates for the next MDS criteria for prodromal PD.

In conclusion, poor cognitive functioning is associated with increased risk of developing parkinsonism, even at intervals greater than eight years, and can be considered a prodromal sign of PD.

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Chapter 7.3

Sleep

ABSTRACT

Sleep disturbances may signal presence of prodromal parkinsonism, including Parkinson's Disease (PD). Whether general sleep quality or duration in otherwise healthy individuals is related to the risk of parkinsonism remains unclear. We hypothesized that both worse self-reported sleep quality and duration, as well as a longitudinal deterioration in these measures, are associated with the risk of parkinsonism, including PD. In the prospective population-based Rotterdam Study, we assessed sleep quality and duration with the Pittsburgh Sleep Quality Index in 7,726 individuals (mean age 65 years, 57% women) between 2002-2008, and again in 5,450 individuals between 2009-2014. Participants were followed until 2015 for a diagnosis of parkinsonism and PD. Outcomes were assessed using multiple modalities: interviews, physical examination, and continuous monitoring of pharmacy records and medical records of general practitioners. We used Cox regression to associate sleep, and changes in sleep over time, with incident parkinsonism and PD, adjusting for age, sex, education and smoking status. Over 64,855 person-years in 13 years of follow-up (mean: 8.4 years), 75 participants developed parkinsonism, of whom 47 developed PD. We showed that within the first 2 years of follow-up, worse sleep quality (hazard ratio 2.38 per standard deviation increase (95% confidence interval 0.91-6.23)) and shorter sleep duration (hazard ratio 0.61 per standard deviation increase (0.31-1.21)) related to a higher risk of parkinsonism. Associations of worse sleep quality (hazard ratio 3.86 (1.19-12.47)) and shorter sleep duration (hazard ratio 0.48 (0.23-0.99)) with PD were more pronounced, and statistically significant, compared to parkinsonism. This increased risk disappeared with longer follow-up duration. Worsening of sleep quality (hazard ratio 1.76 per standard deviation increase (95% confidence interval 1.12-2.78)), as well as shortening of sleep duration (hazard ratio 1.72 per standard

deviation decrease (1.08-2.72)), were related to PD risk in the subsequent 6 years. These results suggest that deterioration of sleep quality and duration are markers of the prodromal phase of parkinsonism, including PD.

Chapter 7.3

BACKGROUND

Parkinson's Disease (PD) is primarily characterized by motor disturbances,[1] but also includes non-motor features. Sleep-wake disturbances are a common non-motor feature of PD[2-8] and related synucleinopathies.[9-11] Sleep-wake disturbances are also reported to precede a diagnosis of parkinsonism in prodromal PD.[12] Objectively measured increases in sleep fragmentation have also been related to increased PD pathology at brain autopsy in old individuals without PD.[13] Sleep-wake disturbances may be a risk factor for PD, or indicate presence of disease in a prodromal phase.[14,15]

Several sleep disorders have been reported to precede PD or related synucleinopathies,[12] including rapid eye movement (REM) sleep behavior disorder[1,16,17] and obstructive sleep apnea.[18-21] These seem to represent, however, only the 'tip of the iceberg' of various sleep-wake disturbances in prodromal PD.[22-27] Subclinical impairments in sleep, such as poor sleep quality and short sleep duration, are more common in the general population and may well capture aforementioned sleep-wake disturbances. These impairments are particularly important as they are often investigated and easily determinable aspects of sleep in any healthcare setting. To date, however, only few studies investigated if sleep duration reflects prodromal PD,[27,28] and none studied sleep quality. Furthermore, it is unknown if long-term changes in sleep duration and quality relate to subsequent risk of parkinsonism, including PD.

We studied the association of subjectively assessed sleep quality and duration with parkinsonism, including PD. We hypothesized that i) worse sleep quality, and shorter sleep duration, are associated with the risk of parkinsonism, including PD; and ii) deterioration in sleep quality and duration over time is associated with the

subsequent risk of parkinsonism. We tested these hypotheses in a prospective, population-based study, using the Pittsburgh Sleep Quality Index to (repeatedly) measure sleep quality and duration, with up to 13 years of follow-up for incident parkinsonism.

METHODS

Study setting

The study was embedded in the Rotterdam Study, a large, prospective, population-based study in a suburban district in the city of Rotterdam, the Netherlands, details of which are described elsewhere.[29] The study was set up to investigate the frequency, risk factors and natural history of common chronic diseases in the elderly, including neurodegenerative diseases such as PD. The first cohort was initiated in 1990 and included 7,983 individuals aged ≥55 years, was expanded with 3,011 individuals aged ≥55 years in 2000, and further expanded with 3,932 individuals aged ≥45 years in 2006. Examination rounds consisted of a home interview and visits to our dedicated research center, including a wide range of questionnaires and physical measurements. Visits are repeated every 4-5 years. Measurements are kept similar across inclusion rounds and time. In between examination rounds, incident disease is assessed with continuous linkage of the study database and medical records of general practitioners, which also holds summaries from all specialist and inpatient care.

Study population

We included participants from all three inclusion rounds when a sleep questionnaire, the Pittsburgh Sleep Quality Index (PSQI), was first introduced. At this baseline visit (between 2002-2008), we included 7,726 individuals who had valid data on sleep quality or sleep duration, did not have prevalent parkinsonism or PD,[30] and were not cognitively impaired based on a Mini-mental state examination (MMSE) score > 25. We followed the remaining participants until the first of: onset of parkinsonism or PD, 1 January 2015, or death. Study follow-up for incident parkinsonism was nearly complete (64,855 person-years [98.1%]).[31]

For analyses of changes in sleep over time, we similarly included 5,450 individuals at the follow-up visit (between 2009-2014) and started follow-up time for parkinsonism and PD after this visit. See *Online Supplementary* Fig. 1 for a detailed flowchart of included participants for analyses at baseline and the follow-up visit.

Assessment of sleep

Subjective aspects of sleep were measured with a Dutch version of the PSQI, which assesses past month's average sleep quality. The PSQI[32] has a good test-retest reliability and validity in a non-clinical sample of older adults.[33] Answers can be categorized, scored and combined into seven component scores ranging from 0 (not problematic) to 3 (very problematic), labeled 'quality', 'latency', 'duration', 'efficiency', 'disturbances', 'sleep medication', and 'daytime dysfunction'. These scores are summed to provide the global PSQI score (range: 0 – 21) of

subjectively assessed sleep quality (hereafter: 'sleep quality'). Higher scores indicate poorer sleep, and scores > 5 indicate a 'poor' sleep quality.

For participants with more than one PSQI component missing, the global PSQI score was not calculated (n=156, 2%). To minimize loss of participants, we calculated weighted component scores for participants who missed one component score (n=1,099, 13%) by multiplying the six-component sum scores by 7/6. Most of these participants missed information on sleep disturbances (n=847) due to introducing a skip rule in PSQI items on disturbances (5a-5j[32]) in a subset of participants, to limit participant burden. If answers to items 5a-5b were both negative ('not in the last month'), items 5c-5j were skipped. Weighting scores minimized any effect of the skip rule on global PSQI scores, as in individuals who answered items 5a-5b negatively, weighted scores were not different between those who followed the skip rule versus those who did not. Analogously, at follow-up we did not calculate the global PSQI score for 484 (8%) due to missing more than one PSQI component at the follow-up visit (and excluded participants who missed global PSQI score at the baseline visit so that changes could not be calculated [n=203]). We weighted scores for 252 participants (5%) who mostly missed data on efficiency (n=190; see flowchart in *Online Supplementary* Fig. 1).

Assessment of parkinsonism and Parkinson's Disease

A detailed description of assessment methods is provided in **Chapter 4.1**. In short, we used four overlapping modalities to screen for potential parkinsonism: in-person examinations (on average every 4 years), in-person interviews, use of antiparkinson medication, and clinical monitoring alerts.

Of all individuals who screened positive in any of these methods, complete medical records (including letters from medical records of specialists and general practitioners) were studied and case reports were drawn up covering all potentially relevant information to establish presence and cause of parkinsonism. These case reports were evaluated by a panel led by an experienced neurologist.

Parkinsonism was defined by presence of hypo- or bradykinesia in combination with ≥1 cardinal sign (resting tremor, rigidity or postural imbalance) observed by any physician, or a clinical diagnosis of parkinsonism by a neurologist or geriatrician (if motor examination details were unavailable). PD was only diagnosed after exclusion of secondary causes, in individuals with at least one of: 1) a clinical PD diagnosis by a neurologist or geriatrician; 2) positive response to dopaminergic treatment. Individuals with parkinsonism who did not fulfill PD criteria were considered secondary parkinsonism cases. After initial diagnosis, medical records of all incident parkinsonism cases continued to be scrutinized until the end of the study period for new information that could lead to a revision of the diagnosis.

Potential confounders and effect-modifiers

Analyses were adjusted for potential confounders measured at baseline, selected based on relevant publications[1,22,34]: age, sex, education and smoking history. Educational attainment was assessed by interview and categorized as primary, secondary/lower vocational, intermediate vocational, and higher vocational or university. Smoking habits were assessed by interview and categorized as never, former or current smoking. We also examined potential effect-modification by depressive symptoms and anxiety disorders. Depressive symptoms were assessed

with the validated Dutch version[35] of the Center for Epidemiological Studies Depression Scale[36]. Presence of an anxiety disorder was assessed by an adapted version of the Munich Composite International Diagnostic Interview[37].

Statistical analysis

A detailed explanation of our statistical methods is provided in the *Online Supplementary* Text. In short, we first used Cox proportional hazards regression models to associate both sleep quality and duration at baseline with both incident parkinsonism and PD. As we found that the Cox model assumption of proportionality was violated in some analyses, we also examined how aforementioned associations changed over follow-up time by performing analyses in incremental epochs of follow-up time from baseline (extending follow-up time e.g. baseline to 2 years, baseline to 4 years, etc.)[38], or using a stratified Cox model to obtain period-specific hazards (e.g. baseline to 2 years, 2 to 4 years, etc.). We furthermore looked at the effect of other PSQI components separately. As sensitivity analyses, we restricted analyses to individuals without comorbid depression and anxiety. We also investigated potential effect-modification by age, sex, and presence versus absence of any of four parkinsonian signs. Second, we related changes in sleep quality and duration between the baseline and the follow-up visit with incident parkinsonism and PD after the follow-up visit.

Variables were standardized and, when right-skewed, log-transformed before standardization. Missing values on covariates were imputed using five multiple imputations.

RESULTS

Population characteristics

Characteristics of the study sample at baseline are summarized in *Table 1*. Median global PSQI score was 3, and 2,115 participants (27%) scored over 5 indicating poor sleep quality. Global PSQI score and sleep duration were moderately correlated (Spearman's r = -0.69; p<0.01)). During 13.0 years of follow-up (mean 8.4 years), we observed 75 incident parkinsonism cases, of which 47 (63%) with PD (*Online Supplementary Table 1*).

Analyses using overall follow-up

Sleep quality was not associated with the risk of parkinsonism (hazard ratio [HR] per standard deviation [SD] increase in global PSQI score: 0.95 (95% confidence interval (CI) 0.76-1.20)) or PD (HR per SD increase 0.87 (95% CI 0.65-1.16)). We observed similar estimates when analyzing categorized poor (versus good) sleep quality: HR 0.97 (95% CI 0.57-1.66) for parkinsonism, and HR 0.79 (95% CI 0.39-1.59) for PD (*Online Supplementary Table 2*).

Longer sleep duration was not associated with the risk of parkinsonism (HR per SD increase 1.21, 95% CI 0.95-1.54) and PD (HR 1.24, 95% CI 0.92-1.69). After categorizing sleep duration, we did not observe a significant increase in risk with increasing categories of sleep duration (*Online Supplementary Table 2*).

In aforementioned analyses for PD risk, but not for parkinsonism, the proportionality assumption for both sleep quality and duration was significantly violated.

Table 1 | Study population characteristics.

Characteristic (unit)	Total sample	Incident PS	No incident PS
	N = 7,726	N = 75	N = 7,651
Age at baseline (years)	65.4 ± 10.3	71.6 ± 8.4	65.4 ± 10.3
Female	4,396 (57%)	33 (44%)	4,365 (57%)
Educational level			
Primary education	708 (9%)	8 (11%)	700 (9%)
Lower/intermediate or lower vocational	3,088 (40%)	29 (39%)	3,060 (40%)
Higher or intermediate vocational	2,371 (31%)	24 (32%)	2,347 (31%)
Higher vocational or university	1,559 (20%)	14 (19%)	1,545 (20%)
Smoking status			
Never	3,416 (44%)	34 (45%)	3,383 (44%)
Former	3,549 (46%)	33 (44%)	3,516 (46%)
Current	761 (10%)	8 (11%)	753 (10%)
Cognitive functioning (MMSE score)	28 (27-29)	28 (27-29)	28 (27-29)
Depressive symptoms (CES-D score)	3 (1-8)	4 (1-8)	3 (1-8)
Presence of any anxiety disorder	588 (8%)	8 (11%)	580 (8%)
Presence of any parkinsonian signs	807 (10%)	16 (21%)	792 (10%)
Sleep quality (global PSQI score)	3 (2-6)	3 (1-6)	3 (2-6)
Missing	46 (1%)	0 (0%)	46 (1%)
Sleep duration (hours)	6.8 ± 1.2	7.1 ± 1.3	6.8 ± 1.2

Characteristics of study population at baseline. Values are expressed as frequency (%) for categorical variables and mean ± standard deviation or median (interquartile range) for continuous variables, unless specified otherwise. Includes imputed values for covariates. CES-D=Center for Epidemiological Studies – Depression Scale; MMSE=Mini-mental state examination; N=sample size; PS=parkinsonism; PSQI=Pittsburgh Sleep Quality Index.

Studying different epochs of follow-up time

We found that worse sleep quality related to an increased risk of parkinsonism (HR 2.38, 95% CI 0.91-6.23) in the first 2 years of follow-up, which disappeared when increasing follow-up time from baseline (Fig. 1A). In these 2 years, associations were more pronounced, and statistically significant, for PD (HR 3.86, 95% CI 1.19-12.47) compared to parkinsonism. Results for sleep duration were analogous (Fig. 1B): short sleep duration was associated with an increased risk of parkinsonism (HR 0.61, 95% CI 0.31-1.21) and PD (HR 0.48, 95% CI 0.23-0.99). Additionally, analysis of period-specific hazard ratios using a stratified Cox model suggested that associations of worse sleep quality, and shorter sleep duration, with an increased risk of parkinsonism and PD are confined to the first 2 years of follow-up (*Online Supplementary Fig. 2*).

Other PSQI components

Most PSQI components showed a similar pattern of associations with cumulative increasing follow-up duration, except for sleep medication (Fig. 2A-F). We observed noteworthy changes in effect sizes from short to long follow-up for sleep efficiency, and to a lesser extent for sleep quality, latency and daytime dysfunction (Fig. 2A-C; Fig. 2F; *Online Supplementary Table 3*). Also, for daytime dysfunction, the direction of hazard ratio estimates changed over increasing epochs of follow-up (*Online Supplementary Table 3*).

Sensitivity analyses

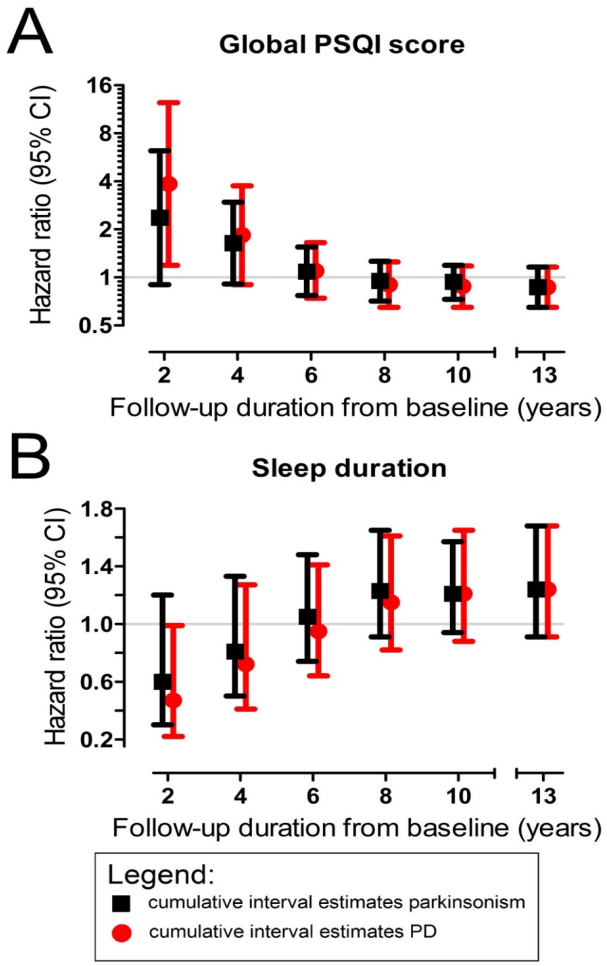
We further restricted the sample to individuals without clinically relevant depressive symptoms and without any anxiety disorder, leaving 6,605 individuals of which 61 cases of parkinsonism, including 39 cases with PD. Associations over cumulatively increasing follow-up duration were similar to the total sample (*Online Supplementary* Fig. 3). For the association of sleep duration with PD, all hazard ratios shifted to higher values. As a result, longer sleep duration was now associated with increased PD risk in the overall follow-up (HR 1.47, 95% CI 1.02-2.11), for which proportionality was not violated.

Stratified analyses

Analyses stratified at median age did not reach statistical significance. We observed hazard ratio estimates suggesting associations of worse sleep quality with a lower risk of parkinsonism and PD in younger individuals, while hazard ratios in older individuals were close to the null. Similarly, estimates also suggested associations of longer sleep duration with a higher risk of both outcomes in younger individuals. Case numbers in separate strata were small. Also, there were no significant interaction terms between age and sleep quality or duration on the risk of either outcome (*Online Supplementary Table 4*).

We observed a similar relation between sleep quality and duration and disease risk in individuals without parkinsonian signs at baseline. Statistically testing these interaction terms on a multiplicative scale showed significant interaction terms of sleep quality with presence of parkinsonian signs on the risk of both parkinsonism and PD (*Online Supplementary Table 4*).

Figure 1 | Associations of sleep quality and duration with risk of parkinsonism and Parkinson's Disease, per cumulatively increasing duration of follow-up.



The associations of (A) sleep quality and (B) sleep duration with incident parkinsonism and PD are shown for cumulatively increasing follow-up duration within the study timeframe. Hazard ratio estimates were obtained from multivariate Firth's penalized Cox regression models by censoring all participants still at risk at year 2, 4, 6, 8 and 10 after baseline, and after the total follow-up of 13 years. Hazard ratio estimates were adjusted for age at baseline, sex, educational level and smoking status, are expressed per standard deviation increase of (A) worse sleep quality, or (B) longer sleep duration, and are plotted at a (A) logarithmic (base 2) scale and (B) a linear scale. CI=Confidence Interval; PD=Parkinson's Disease; PSQI=Pittsburgh Sleep Quality Index

Change in sleep quality or duration

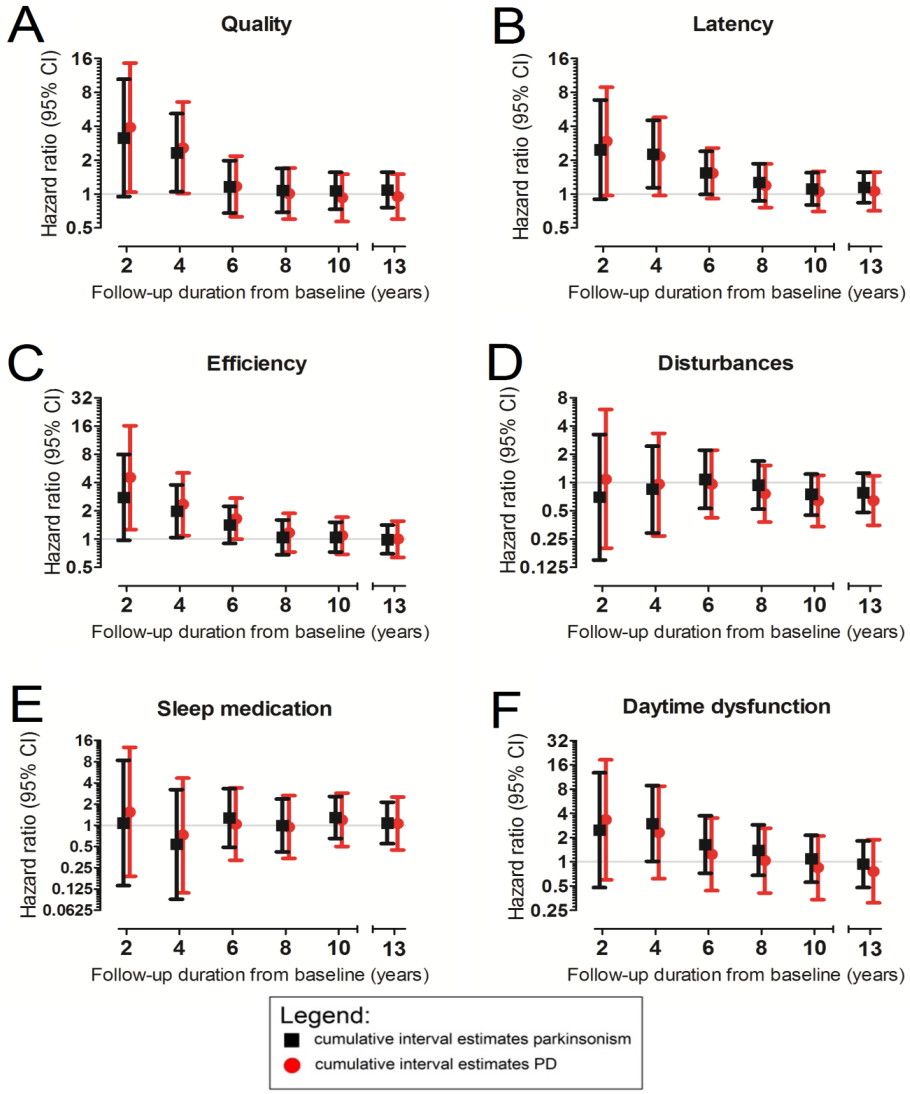
Characteristics of the study population at the follow-up visit are provided in *Online Supplementary Table 5*. Changes in sleep between the baseline and follow-up visit were measured over 10.9 years (on average 6.0 years) in all participants. In the subsequent 6.0 years (average follow-up: 2.9) after the follow-up visit, we observed 25 incident parkinsonism cases, of which 17 with PD. Worsening of sleep quality was related to a subsequent increase in PD risk (HR per SD increase 1.76, 95% CI 1.12-2.78), as was a shortening of sleep duration (HR per SD increase 1.72, 95% CI 1.08-2.72; *Table 2*). Results were independent of the absolute average level of sleep quality or duration (*Table 2*).

Table 2 | Association of changes in sleep quality and duration between the baseline and follow-up visit, and risk of parkinsonism and Parkinson's Disease.

Determinant (unit)	Parkinsonism		Parkinson's Disease	
	Cases/N	HR (95% CI)	Cases/N	HR (95% CI)
Change in sleep quality (worse sleep)	25/5206	1.23 (0.83-1.83)	17/5244	**1.76 (1.12-2.78)**
Change in sleep duration (shorter sleep)	25/5244	1.45 (0.99-2.13)	17/5238	**1.72 (1.08-2.72)**

Changes in sleep quality were modeled per standard deviation increase ('worsening') of global PSQI score, and changes for sleep duration were modeled as standard deviation decrease ('shortening') of sleep duration from the baseline visit to the follow-up visit. Hazard ratio estimates are adjusted for age at baseline, sex, educational level, smoking status and time interval between measurements. Additional adjustment for depressive symptoms at baseline had no substantial effect on point and interval estimates. After additional adjustment for the average level of sleep quality or sleep duration of the two measurements, point and interval estimates for the relation with parkinsonism barely changed. Estimates for associations of change in sleep quality (HR 1.87, 95% CI 1.12-3.10) and change in sleep duration (HR 1.85, 95% CI 1.14-2.98) with risk of Parkinson's Disease increased. Bold estimates indicate statistically significant results at p<0.05. CI=Confidence interval; HR=Hazard ratio; N=Sample size.

Figure 2 | Associations of Pittsburgh Sleep Quality Index component scores with risk of parkinsonism and Parkinson's Disease, per cumulatively increasing duration of follow-up.



The associations of the PSQI components (A) quality, (B) latency, (C) efficiency, (D) disturbances, (E) sleep medication, and (F) daytime dysfunction with incident parkinsonism and Parkinson's Disease are shown for cumulatively increasing follow-up duration within the study timeframe. CI=Confidence interval; HR=Hazard ratio; N=Sample size; PD=Parkinson's Disease; PSQI=Pittsburgh Sleep Quality Index.

Also, additional adjustment for depressive symptoms at baseline did not attenuate results. Associations of sleep quality (HR 1.23, 95% CI 0.83-1.83) and sleep duration (HR 1.45, 95% CI 0.99-2.13) with incident parkinsonism were less pronounced. When examining hazard ratios over increasing epochs of follow-up time measured from the follow-up visit, we found that worsening of sleep quality, and shortening of sleep duration, were associated with parkinsonism on the short term, but not the longer term (*Online Supplementary* Fig. 4). For both sleep parameters, risk of PD was also slightly higher on the short than on the long term.

DISCUSSION

In the general population, baseline sleep quality and duration within the next 2 years relate to incident parkinsonism, and specifically to PD. Similarly, deterioration over 6 years in these parameters is associated with incident parkinsonism and PD.

Several methodological considerations should be mentioned. First, our study focused on subjectively measured sleep, which do not necessarily reflect the same sleep constructs as objective measurements. While subjective measures reflect the experience of sleep, objective measurements indicate physiological sleep. Therefore, subjective measures cannot provide similar insight in underlying biological processes as objective measures (e.g. polysomnography). Second, we deliberately excluded individuals with cognitive impairment to minimize the potential for information bias on sleep quality[39,40] and duration,[39,41] however, as individuals with cognitive impairment are at increased risk of parkinsonism[42] we cannot rule out that that exclusion of those individuals led to some underestimation of true associations of sleep quality and duration with incident

parkinsonism. In addition, individuals with cognitive impairment are also predisposed to develop REM sleep behavior disorder,[43] which has been suggested to be associated to a longer sleep duration in the general population.[44] This could lead to an underestimation of associations of sleep duration with parkinsonism and PD. Third, although the PSQI is used in patients with PD,[45] it may miss PD-specific sleep disturbances.[46,47] Patients with prodromal disease may thus underreport sleep problems, or overstate their sleep quality. If so, we have even underestimated especially short-term effect estimates of worse sleep quality with increased risk of parkinsonism and PD risk. Fourth, the number of parkinsonism and PD cases in our study was relatively small, which may have rendered us unpowered to detect small effects.

We found associations of poor sleep quality and short sleep duration with increased risk of parkinsonism, and especially PD, in the first 2 years of follow-up, attenuating with incremental follow-up. These observations on sleep quality and duration build on findings from registry-based studies in general practice, which previously showed increases in the prevalence of insomnia 2 years[22,48] but not 5 or 10 years before diagnosis of PD.[22] Such results suggest that sleep disturbances occur as prodromal features rather than as causes of PD and related synucleinopathies, as sleep is measured closer to the diagnosis of an incident case when follow-up is short. Our measurements of sleep likely represent common, subclinical sleep problems as well as those severe enough to diagnose a sleep disorder, and therefore fit well with the variety of sleep disturbances preceding PD.[12]

Mechanisms behind sleep disturbances in prodromal PD remain speculative. Sleep may be disturbed by early-stage dysfunction of serotonergic neurons in the dorsal raphe nuclei and sleep-promoting areas in the basal forebrain.[49] Such

dysfunction may also negatively impact switching between sleep and wake [50]. Additionally, early spread of pathology to the coeruleus/subcoeruleus complex may disturb REM sleep independent of REM sleep behavior disorder.[15] Sleep may also be impaired via circadian dysfunction occurring around the time of diagnosis,[2] via hypothalamic neuron loss,[51,52] or via the loss of dopaminergic modulation.[53]

Of note, our results do not rule out that sleep disturbances may be a cause of PD. An effect of sleep disturbance on neurodegenerative disease is plausible, as sleep deprivation has been shown to increase levels of β-amyloid, a pathological hallmark of Alzheimer's Disease. Mechanisms include decreased clearance,[54] or activity-dependent increased production, of β-amyloid. The sleep wake cycle has also been shown to regulate tau levels, and sleep deprivation can increase extracellular levels of tau and, interestingly, α-synuclein.[55] A recent study showed that increased actigraphy-derived sleep fragmentation in old individuals without PD was associated with an increased burden of PD pathology at brain autopsy.[13] This indicates that objective disturbances, besides subjectively impaired sleep, relates to PD pathology. Unfortunately, the cross-sectional design of that study precluded inference on temporality of the association.

Analyses of changes in sleep quality and duration suggest that sleep in prodromal PD already deteriorates over 2 years prior to diagnosis in the general population, independent from baseline depressive symptoms, and the absolute levels over which the changes occurred. To our knowledge, the only study investigating changes in sleep has been performed in patients with REM sleep behavior disorder.[56] That study, however, reported opposite findings: improving insomnia symptoms and increasing self-reported sleep duration was associated with an increased the risk for conversion to PD and dementia with Lewy bodies.

Differences in findings could results from their selection of patients prone to develop a severe, cognitively impaired subtype of prodromal PD,[57] but differences may additionally be explained by non-recognition of sleep problems due to including individuals with subclinical cognitive deficits.[58-60] Their study not only had a high incidence (50%) of Lewy body dementia patients, but also showed underreporting (reporting increased sleep duration and quality discrepant from objective decreases in total sleep time) in those developing neurodegenerative disease.[56]

If aforementioned changes in sleep were driven by a specific sleep disorder, REM sleep behavior disorder may not be a likely candidate: individuals with REM sleep behavior disorder in a population-based polysomnography study had a similar sleep quality, and even longer sleep duration, than others.[44] REM sleep behavior disorder patients also did not perceive their sleep as worse, or shorter, than healthy controls.[56]

After excluding individuals with comorbid depressive symptoms or anxiety disorders in sensitivity analyses, most results remained similar, although hazard ratio estimates of the relation of sleep duration with PD risk were slightly higher. This resulted in an association of longer sleep duration with increased PD risk in the overall follow-up. Given the number of associations investigated in our sensitivity analyses, and the small number of cases when restricting the sample, this result should be considered preliminary and interpreted with caution. One explanation would be that in these sensitivity analyses individuals in a late prodromal phase of PD may have been selectively excluded, as depression and anxiety are both part of the prodromal phase and considered predominantly late features.[22,61,62] This could have resulted in selective exclusion of susceptible individuals[38] resulting in a decreased long-term risk of PD in those remaining

individuals with short sleep duration. It is also possible that short sleep duration is merely symptomatic of (prodromal emergence of) depression, which explains why exclusion of individuals with depression resulted in an inverse association of sleep duration with PD.

Patterns of associations between separate PSQI components and PD risk over time indicate that, aside from diminishing sleep duration, decreasing sleep efficiency may mark prodromal disease. This also applies to sleep quality, latency and daytime dysfunction to a lesser extent. Interestingly, these aspects of sleep may correlate with known markers of prodromal PD such as pain or autonomic failure,[22] or excessive daytime sleepiness.[12,63]

In conclusion, poor sleep quality and short sleep duration increased the risk of parkinsonism and PD in the next 2 years. Moreover, sleep quality and duration change for the worse over 2 years prior to a diagnosis of parkinsonism, especially PD. Both are congruent with presence of prodromal PD progressively deteriorating sleep. Future studies on prodromal PD are needed to investigate associations with objective measures of sleep, and to assess the predictive value of (perceived) shortening or worsening of sleep over known (sleep) markers of prodromal parkinsonism.

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Chapter 8

General discussion

There is no longer room for preventive nihilism.

Albert Hofman et al. Epidemiology of neurological diseases in elderly people: what did we learn from the Rotterdam Study? The Lancet Neurology. 2006 Jun;5(6):545-50.

Chapter 8

OVERVIEW

I used observational data from the general population to obtain novel insight into determinants of motor functioning and the prediagnostic phase of PD. In the next paragraphs, I discuss the main findings of this thesis in a broader clinical context, point out methodological considerations that merit attention in the interpretation of these findings, and offer directions for future research.

UNRAVELLING DETERMINANTS OF MOTOR FUNCTIONING

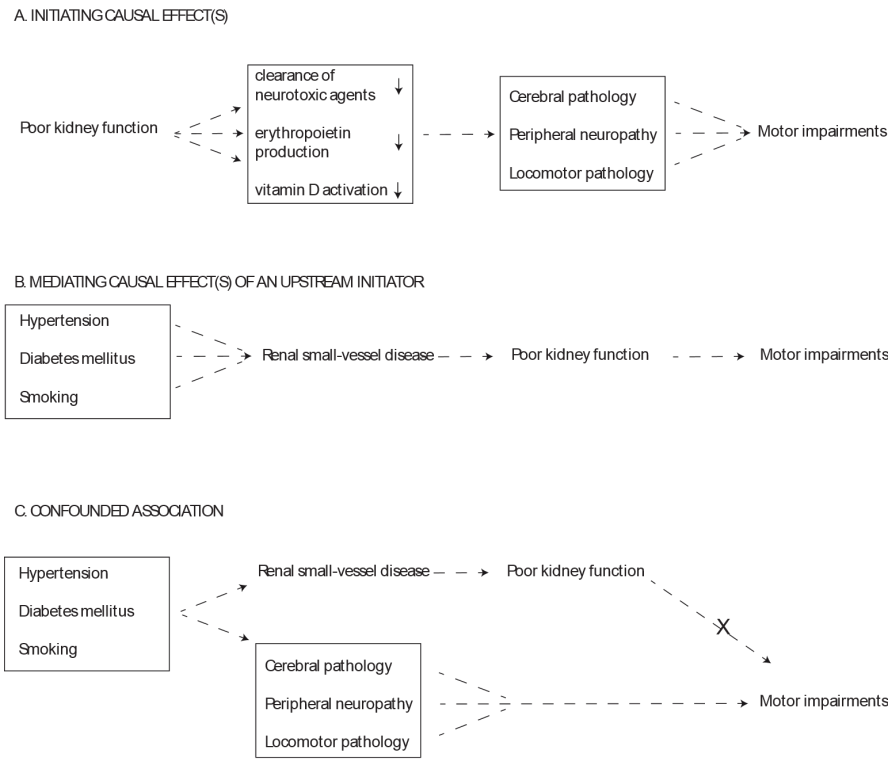
Clinical context

Adequate motor performance is a key prerequisite for functional independence, in particular among elderly.[1,2] Given the ageing of populations worldwide, prevention of motor impairments among older adults may have broad public health implications, highlighting the importance of unravelling determinants of motor functioning. In **Chapter 2** of this thesis, I obtained novel insight into genetic, cerebral microstructural and metabolic determinants of motor functioning among individuals without a clinical neurodegenerative disease. However, several key gaps in knowledge remain.

First, insight on which genetic variants affect motor functioning, and the mediators of their effects, remains relatively scarce. While I focused on genetic variants implicated in PD in **Chapter 2.1**, it is likely that genetic variants implicated in other diseases characterized by gait impairments also affect gait in individuals without clinical disease. Second, it remains to be elucidated which underlying pathologies drove the associations between cerebral microstructural determinants and motor functioning in **Chapter 2.2**. Subtle impairments in motor

functioning are an overlapping prodromal feature across several neurodegenerative diseases (**Chapter 3**). To what extent this overlap reflects shared pathophysiological mechanisms is currently not clear. Third, the biological underpinnings of associations of metabolic markers such as poor kidney (**Chapter 2.3**), thyroid[3] or liver function[4] with motor functioning remain largely unclear. In *Box 1*, I illustrate several mechanisms that these associations may reflect, using poor kidney function as an example.

Box 1 | Kidney function and motor impairments: putative underlying mechanisms.



The association between kidney function and motor impairments may reflect one or more of the following effects of kidney function:
A. initiating causal effect, that could be mediated by reductions in clearance of potentially neurotoxic agents, erythropoietin production, or vitamin D activation;
B. mediating causal effect of an upstream initiator such as high blood pressure;
C. confounded association (e.g., non-causal proxy for other pathology with shared initiators).

In the coming years, observational studies with serial assessments on metabolic markers, potential mediators and motor functioning can leverage novel statistical methods[5] to quantify how much each of these mechanisms explains the association. Ultimately, an improved understanding of the underlying biology will facilitate the development of interventions that can be applied in a clinical setting.

Methodological considerations on how to move forward

The slow progress in the discovery of determinants of motor functioning highlights the impact of several methodological issues: nature of assessments, outcome definition, and the impact of brain reserve.

As for the nature of assessments, routine motor examinations in neurological or geriatric clinics currently include a qualitative assessment of movement in different body parts to determine the presence of parkinsonian signs. For instance, the presence of brady- or hypokinesia is assessed by qualitatively rating facial expressivity, arm swing amplitude, finger tapping and step length. This approach is prone to interrater variability, e.g. a patient's lower movement bradykinesia may be 'mild' to one clinician and 'moderate' to another, and - given the qualitative nature of examinations- also ignores substantial residual variability in clinical severity beyond 'mild', 'moderate' or 'severe'. If inaccurate classification of motor functioning is independent of exposure to a putative determinant, it introduces non-differential misclassification. If classification of motor dysfunction is associated with exposure to a putative determinant, it may introduce information bias. Both types of information error may hamper the discovery of true associations between determinants and motor functioning. This is especially concerning for putative risk factors that are strongly associated with age (e.g.

vascular risk factors), as the extent to which motor dysfunctions are interpreted as 'physiologic signs of ageing' varies strongly between clinicians. In the coming years, these shortcomings can be overcome by implementing quantitative methods to assess motor functioning on a broad scale, in research as well as in clinical settings.

As for outcome definition, it is noteworthy that the primary outcome of the largest GWAS on gait to date, which failed to identify any implicated genetic variants at genome-wide significance level, was gait speed.[6] Gait speed may be influenced by a heterogeneous array of neurological and non-neurological factors, rendering effect sizes of genetic variants predisposing to one of these factors very small. By contrast, genetic discovery studies of specific motor functions may unmask genetic variants genetic (e.g., genetic discovery studies on shuffling may uncover genetic variants implicated in PD). The advance of wearable sensors will facilitate minimally burdensome assessments of motor functioning on an even broader spectrum of specific motor functions in years to come.[7] This would be similar to the field of cognitive functioning, in which GWAS on diverse cognitive domains (e.g., memory, executive functioning) have uncovered task-specific genetic effects.[8,9] From an analytical point of view, the precision of motor functioning assessment can be further refined by leveraging raw data rather than aggregate measures. For instance, data from electronic gait assessments could be analyzed at the level of each step, or even at the level of each single recorded pixel of a step, instead of using aggregate measures of a recorded walk. This will increase statistical power to detect associations. Another issue that could improve statistical power is the sample size of studies on determinants of motor functioning. Compared to the field of cognitive functioning, previous motor studies had a very small sample size; for instance, the

largest GWAS on cognitive functioning, which identified 148 implicated genetic variants, included roughly ten times as many participants compared to the largest of GWAS on gait (~300,000 vs. ~30,000). Therefore, increasing the number of participants within studies on motor functioning and combining data across studies is a key priority. In *Box 2*, a potential approach that could facilitate cross-study analyses is presented.

Box 2. Global motor functioning.

Currently, substantially different sets of quantitative motor parameters are assessed across studies, precluding replication of key findings or meta-analyses of results. In the field of cognition, a similar challenge was overcome by the identification of a cognitive 'g factor', which is effectively interchangeable across different cognitive test batteries, provided that each battery encompasses a diverse combination of tests that represent the broad spectrum of cognitive functions.[10,11] Similarly, if a motor g factor could be estimated accurately using different combinations of quantitative motor parameters, it would be possible to compare results on global motor across studies that measured different sets of quantitative motor parameters. In the coming years, observational studies with diverse, deep motor phenotyping will assess whether a g factor could be identified across different combinations of diverse quantitative motor parameters.

Another key consideration is that most observational studies on putative determinants of motor functioning to date have not taken into account a potential effect-modifying influence of 'brain reserve'. This concept indicates that

the threshold under which neuropathology evokes (sub)clinical manifestations varies between individuals by the brain's capacity to compensate, i.e. individuals with higher brain reserve can remain asymptomatic longer than others even if faced with the same extent of neuropathology.[12] Brain reserve is affected both by maximally attained brain development, which in the cognitive functioning field is often referred to as 'cognitive reserve' and typically estimated by assessing the highest education attained, as well as by the extent of (prediagnostic) neuropathology or neurodegeneration. While the concept of brain reserve has been a key focus of cognitive functioning research in recent decades,[13] it has attracted far less attention in motor functioning research. Still, there is some evidence to suggest that individuals with higher brain reserve are less susceptible to the effects of white matter lesions on motor functioning,[14] and some studies suggest that head trauma may worsen phenotypical severity in PD, possibly even uncovering latent pathology (i.e., unmask prediagnostic PD patients).[15] In the absence of direct markers of brain reserve, prodromal features could be used as proxies to estimate its potential effect-modifying influence on associations between putative determinants and motor functioning in the context of neurodegenerative diseases. I provide an illustration of this approach in **Chapter 2.1**, by showing that genetic variants implicated in PD affect gait in individuals with below-average cognitive functioning, and also affect cognitive functioning in individuals with below-average gait. Future studies will also assess effect modification of genetic effects on motor functioning by direct markers of specific neuropathologies (e.g., β-amyloidopathy, tauopathy, α-synucleinopathy, small-vessel disease).

Chapter 8

UNRAVELING THE UNDERLYING MECHANISMS OF PARKINSON'S DISEASE

Clinical context

In **Chapter 4.1**, I observed a decline in the incidence of PD in the Rotterdam Study between 1990 and 2011. My observation was in line with two studies in the UK and Taiwan.[16,17] By contrast, the incidence rate of PD in a study in the United States declined between 1976 and 2005,[18] and mortality associated with PD and related diseases rose in England between 2001 and 2014 (**Chapter 4.2**). These differential temporal trends highlight the need for insight into determinants and underlying mechanisms of PD. Specifically, three topics warrant consideration: variability in the evolution of prodromal features, spread of α-synucleinopathy, and the effects of contributing neuropathologies.

As for the evolution of prodromal features in the prediagnostic phase of PD (**Chapter 7.1**), it is important to note that I focused on group norms. Previous studies have observed differences in prognosis between PD patient subgroups that were defined by demographics or clinical features at the time of PD diagnosis.[19-21] These observations have inspired etiologic studies to assess whether subgroup differences in clinical phenotype are a consequence of differences in underlying mechanisms, however, such investigations have been complicated by the advanced stage of pathology in patients with clinical PD. By contrast, pathological processes are not yet as advanced in these individuals as in clinical PD patients. Once identified, subgroup differences in in the evolution of prediagnostic features may shed light on underlying mechanisms of PD in the prediagnostic phase. In *Box 3*, I provide an approach through which this gap in clinical knowledge could be addressed.

Box 3. Genetic effect modification of prediagnostic trajectories in Parkinson's Disease.

PD patients with high genetic susceptibility for PD are generally diagnosed with clinical PD at a younger age than other PD patients,[22] suggesting that differences in the underlying pathophysiology by genetic susceptibility may affect the duration of the prediagnostic phase of PD. Building on my observation that prediagnostic PD patients increasingly report problems in basic daily activities (BADL) in the last five years before clinical diagnosis (**Chapter 7.1),** I hypothesized that the slope of decline in BADL may be steeper in prediagnostic PD patients who have high genetic susceptibility for PD than in others.

To explore this hypothesis, I modelled a genetic risk score for PD (see **Chapter 5.1**), BADL and an interaction term of the genetic risk score with BADL as independent variables and PD as the dependent variable. Interestingly, I observed that prediagnostic PD patients with high genetic predisposition tend to decline more steeply in BADL than other prediagnostic PD patients (p for interaction term = 0.007). As a result, the prodromal BADL impairment interval (i.e., the period in which patients and matched individuals differ significantly) is shorter in PD patients with high genetic predisposition to PD than in other PD patients. These data suggest that prediagnostic pathology may progress faster in PD patients with high genetic susceptibility than in other in PD patients. Replication of these findings is warranted, and future studies may also examine to what extent differences in prediagnostic evolution between patient subgroups correspond with differences in (postdiagnostic) prognosis.

In current clinical practice, PD is often referred to as a 'clinical α-synucleinopathy'. This designation stems from findings in neuropathological studies, which show that PD is primarily characterized by α-synuclein accumulation and aggregation.[23] Although there is currently no empirical evidence in humans that experimentally reducing α-synuclein slows or halts progress of PD, observations from various other lines of research shift the burden of proof to non-causal explanations for the link between α-synucleinopathy and PD (e.g., that α-synuclein is merely a waste product of a different causal pathological process of PD). For instance, α-synucleinopathy strongly correlates with most motor dysfunctions in PD as well as with some prominent non-motor features in PD (e.g., REM sleep behaviour disorder and impaired olfactory function).[24] Furthermore, genetic variants that determine α-synuclein levels and folding are heavily implicated in PD.[22,24] In addition, experimental in vitro and animal models support the notion that lowering α-synuclein could slow PD.[25] In individuals with clinical α-synucleinopathies such as PD, α-synuclein misfolds and forms pathological fibrils that gradually spread across the nervous system. Interestingly, differences in the type and distribution of misfolding can lead to distinct phenotypical clusters, which we currently recognize as PD, dementia with Lewy Bodies or Multiple System Atrophy.[26,27] Even within PD patients there may be more than one route of spread of PD pathology, and different routes may correlate with specific early non-motor phenotypes.[28] This suggests that there may be several biologically distinct PD subtypes, which in turn may be driven by differences in genetic susceptibility (*Box 3*).

Interestingly, α-synucleinopathy only partly explains non-motor phenotypical differences between PD patients, in particular differences in cognitive functioning.[29] In **Chapter 7.2** I showed that subtle cognitive decline may start as

early as six years before parkinsonism diagnosis in PD patients. Interestingly, the duration of parkinsonism before dementia may be associated with different patterns of pathology in PD: patients who develop dementia in the first years after parkinsonism diagnosis generally have a mix of β-amyloidopathy and α-synucleinopathy, whereas patients with longstanding parkinsonism prior to dementia tend to have more pure α-synucleinopathy.[30] In fact, a recent study showed that CSF $A\beta_{42}$ and low caudate uptake on dopamine transporter imaging are both strongly associated with subsequent cognitive dysfunction in patients with clinical PD.[31] Aside from α-synucleinopathy and β-amyloidopathy, cerebral small-vessel disease and possibly also tauopathy may further contribute to cognitive dysfunction in PD, although current empirical evidence on the role of each of these pathologies is scarce.[29,32] In this context, the removal of dementia before parkinsonism as an exclusion criterion for PD in the 2015 MDS clinical diagnostic criteria[33] is a critical development that will encourage future observational studies aiming to quantify the contribution of various pathologies to cognitive decline in PD to include patients irrespective of their cognitive status.

Methodological considerations on how to move forward

Current knowledge on the underlying mechanisms of PD is based in large part on cross-sectional data,[34,35] which make it difficult to definitively establish temporal relationships on underlying causal chains for PD. In the coming years, prospective studies with serial assessments of putative initiators, mediators and markers of PD pathology, both inside the central nervous system and outside of it (e.g., enteric nervous system,[36] skin[37]) will be initiated in individuals who are at high risk of PD.

Such studies will likely shed light on the role of mediators and the influence of multiple neuropathologies on each other in the prediagnostic phase of PD.

Putative mediators of the effects of initiators can be classified in accordance with their presumed place along a causal axis of PD. High upstream mediators may include RNA transcripts that affect pathological processes such as mitochondrial dysfunction, oxidative stress, inflammation or lysosomal dysfunction.[38] These may induce manifestations of α-synucleinopathy or of other pathologies such as β-amyloidopathy[32]. Downstream mediators include degeneration of cortical or subcortical (micro)structucture, which in turn may lead to cognitive deficits. From an analytical point of view, the (many) effects of inhibitors, mediators and effect modifiers can be disentangled by employing novel statistical methods that allow for quantification of distinct causal pathways.[5]

Another key consideration is to what extent multiple neuropathologies influence each other's effects on PD phenotypes. In this context, there are intriguing observational data in animal studies suggesting that the severity and effects of α-synucleinopathy may be modified by the presence of other pathologies. In particular, in experimental research among transgenic mice overexpressing α-synuclein, those with a coexisting pathology (β-amyloidopathy or tauopathy) had more severe α-synucleinopathy and also worse phenotypes than those without a coexisting pathology.[39,40] If similar mechanisms can be replicated in humans, it would suggest that coexisting pathologies may influence disease progression by rendering target tissues more susceptible to the effects of α-synucleinopathy, even if those pathologies were not causal for PD pathology (i.e., if they had effect-modifying effects, not interactive effects). Within this context, vascular pathology in particular merits attention, since it is highly common among elderly,

is strongly implicated in cognitive functioning in the general population, and embodies a modifiable group of putative effect modifiers.

PUTATIVE RISK AND PROTECTIVE FACTORS OF PARKINSON'S DISEASE

Clinical context

PD was long thought to be a non-genetic disease, however, around the turn of the twenty-first century several rare genetic forms of PD were identified.[41,42] Over the past decade, insight on apparently sporadic PD has increased enormously. Sporadic PD is currently believed to be a multifactorial disease, caused by the effects of multiple genetic variants in combination with lifestyle and environmental factors. To prevent clinical PD, it is vital to causal factors for PD, both genetic and non-genetic, as these may serve as targets for putative preventive trials.

In recent years, tens of common genetic variants that are associated with PD have been identified through hypothesis-free genome-wide association studies (GWAS).[22,43] Each variant is associated with only a modestly altered risk of PD, but in combination these variants can lead to a substantially increased or lowered risk of PD. Approximately 20% of the variation in susceptibility to PD is currently estimated to be due to common genetic variation.[44] Of note, not all of the identified variants are mapped to coding regions of the genome, and the putative causal mechanisms of these variants –or of other variants that they tag– remain largely elusive. Four areas of research warrant particular interest. First, genetic variants identified through GWAS are relatively common. To uncover rarer variants and structural variations and structural variations (e.g., deletions,

duplications, copy-number variants, translocations), there is a need for large genome sequencing studies. Second, the effects of PD risk variants on expression and methylation in various regions across the brain as well as systemically (e.g., in the blood) remain largely elusive. This is a crucial area of research, as some mediators of expression and methylation (e.g., proteins involved in immune response) may be modifiable. In **Chapter 5.2**, I restricted a genetic discovery study to variants that are involved in the regulation of gene expression, specifically, variants in miRNAs as well as miRNA binding sites with PD. I identified associations between 34 miRNA-related variants and PD, including several variants that had previously been implicated in expression of their host genes in the brain. Still, the exact mechanisms by which these variants affect PD risk warrant further investigation. Third, it remains largely unclear which brain (micro)structures serve as downstream mediators of genetic effects. Fourth, empirical data on effects of these variants on prodromal features of PD remain relatively scarce. In **Chapters 2.1 and 3.2.1**, I uncovered effects of these genetic variants on cognition, gait and daily functioning in individuals who were (still) free of clinical PD. Building on these data, future studies will assess effects of genetic variants implicated in PD on brain (micro)structure as well as on potentially modifiable mediators in individuals with prodromal features of PD. Of note, at the time of submission of this thesis, a collaborative study further investigating some of these areas had been submitted to bioRxiv, but had not been peer-reviewed yet.[45]

Putative non-genetic causal factors that may initiate or accelerate PD pathophysiology include exposure to environmental pollutants (e.g., exposure to pesticide[46] or well-water drinking[47]), use of medications (e.g., beta-blockers[47]) or head trauma. In contrast to putative initiators, several putative protective factors

of PD have also been identified, in particular, those related to diet (vitamin B6[48], vitamin E[49], β-carotene[49], fatty acids[50], dairy products[51], coffee,[52] tea[53]), medication (β-2 agonists[54], calcium channel blockers[47], statins[55]) and physical activity (exercise[56]). Furthermore, use of addictive substances such as smoking and caffeine intake may have a preventive or halting effect on pathological processes of PD. Another class of potentially modifiable causal factors are cardiometabolic factors, however, their role as putative non-genetic initiators or accelerators of PD remains equivocal.(*Box 4*)

Box 4. Cardiometabolic factors and Parkinson's Disease.

Studies on the association of individual cardiometabolic factors (e.g., hyperglycemia, hyperlipidemia) with PD have been inconsistent.[57,58] By contrast, a recent registry-based study suggested that these factors may have composite effects on PD, by showing that the metabolic syndrome was associated with the 5-year risk.[59] The study provides reason for careful optimism, since cardiometabolic factors are potentially modifiable, common at the population level, and already serve as targets for broad prevention initiatives aimed at preventing cardiovascular and neurological diseases in the ageing population. Therefore, if these observations would be replicated, especially over longer intervals, they would embody an attractive target for trials aimed at preventing onset of clinical PD in populations at risk.

Of note, it remains uncertain to what extent associations of these non-genetic factors with PD are causal. It also remains largely unclear to what extent putative effects of potentially modifiable factors interact, as insight into composite effects

of these factors on PD risk has been hampered by relatively small sample sizes in single cohorts and the lack of replication across studies. As a consequence, we know little about how much risk of PD could be reduced if multiple putative non-genetic causal factors are targeted simultaneously, and to what extent these potential benefits vary across life-periods, sex or genetic predisposition for PD. As for the latter, my observation of effect modification of the effects of the PREDICT-PD score on PD by a genetic risk score (**Chapter 6.1**) provides reason for cautious optimism, although it is important to note that the PREDICT-PD score not only included putative causal factors, but also prodromal features.

Methodological considerations on how to move forward

To assess causality of observed associations between putative risk or protective factors with PD, several study designs can be considered, each of which has strengths and limitations.

First, the cross-sectional design, which has been used by most association studies on this topic to date. This design can relatively conveniently be executed in a clinical setting, as PD patients are typically already followed-up for clinical care, leaving the task of recruiting unaffected individuals who are willing to have assessments on putative causal factors. However, if the determinant of interest is (indirectly) related to willingness to participate (or withdraw early), this design is susceptible to selection bias as patients are generally more motivated to participate in scientific research into their disease than are unaffected individuals. Furthermore, as putative causal factors are assessed retrospectively, flawed information on exposure to these factors may have a substantial effect on studies using this design. Also, these studies are prone to be affected by reverse

causation, i.e. that prediagnostic PD pathology or predisposition to PD may influence the presence of a factor. In *Box 5*, I discuss the relevance of reverse causation to studies on the association between smoking and the risk of PD.

Box 5. Smoking and the risk of Parkinson's Disease.

Never-smokers have a twofold risk of PD compared to current smokers in observational studies,[47] yet, nicotine trials have failed to show any effect on disease progression.[60,61] There is a similar discrepancy between observational and experimental evidence on the role of caffeine as a putative protective factor for PD.[47,62] This suggests that associations between addictive behavior and PD may actually reflect reverse causation: prediagnostic PD pathology or predisposition to PD may influence addictive behavior. For instance, most individuals who are eventually diagnosed with PD never establish a smoking habit, while those who do and still develop clinical PD tend to quit smoking earlier than their peers in the community.[63]

Prediagnostic PD patients may experience less reward from nicotinic stimulation as a consequence of dopaminergic depletion which is induced by prediagnostic PD pathology; if so, smoking cessation may be a prodromal feature of PD. An alternative –or, perhaps, additional– possible explanation is the 'dopaminergic reserve' theory, i.e. that individuals prone to PD never take up smoking as a consequence of a life-long low dopaminergic state. The converse may explain why artistic professions, which are associated with high dopamine levels and activity, are associated with a substantially reduced risk of PD (**Chapter 6.3**).

Second, the prospective cohort design. Studies using this design are generally less susceptible to selection bias than are cross-sectional studies, provided that there is no selective attrition. Also, as putative causal factors are assessed prospectively, these studies are typically less prone to information error on exposure to these factors than are cross-sectional studies with similar assessment modalities. Yet, these studies may also be affected by reverse causation as well as by residual confounding.[64] Residual confounding is especially a concern for studies on lifestyle-related factors; in particular, individuals with recommended serum levels of vitamins may have other, currently unknown (or insufficiently adjusted) factors that are part of a healthy lifestyle which give them relative protection them against onset of clinical PD.

Third, randomized controlled trials on suppletion (or restriction) of putative causal factors in high-risk individuals. While this design may rule out reverse causation, selection bias (in the absence of selective attrition) and confounding (assuming well-executed randomization), it also has several potential downsides. Given the relatively modest anticipated effect size of these factors on the risk of PD, such studies would require a very large sample size (unless multiple interventions are combined, which would not solve the issue of disentangling independent causal effects). Furthermore, the anticipated effects are strongest in individuals who are relatively early in the prediagnostic phase of PD, suggesting that such studies would require long follow-up, especially given the lack of reliable biomarkers of disease progression as potential intermediate outcome measures. Also, from a broader health perspective, it may be unwise to administer some of these factors with the purpose of potentially reducing the risk of clinical PD, in particular, this would be highly unwanted for smoking.

Fourth, mendelian randomization studies. In this design, genetic variants are used as 'instrumental variables' for a putative causal factor, theoretically minimizing the possible influence of confounding or reverse causality.[65] As a consequence, this approach may facilitate inference of causality of factors that have been associated with PD. It is important to note several assumptions that this approach requires, including -but not limited to: (1) there is an association between the genetic instrument and exposure to the putative causal factor, (2) the genetic instrument affects the outcome (i.e., clinical PD) only through the exposure, and (3) the genetic instrument is independent of factors that confound the exposure-outcome relationship.[66] To date, violation of the first assumption may have hampered the success of mendelian randomization studies on PD.[67] Furthermore, given the complex nature of PD and the clustering of clinical diagnosis in old age, it is important to consider possible biases that may arise as a consequence of time-varying effects of genetic variants.[68] In *Box 6*, I provide an example of the application of a mendelian randomization approach in the Rotterdam Study.

Box 6. Mendelian randomization of serum urate and the risk of Parkinson's Disease.

A Mendelian randomization approach was previously used by other investigators (Simon et al.) to show that higher serum urate concentrations likely have a causal neuroprotective effect on disease progression in individuals with clinical PD.[69] Serum urate concentrations are also inversely associated with the risk of developing PD in the general population.[70] I hypothesized that this may be due to the same neuroprotective effect, since neurodegeneration starts

years before PD is clinically diagnosed.[71] I tested this hypothesis within the population-based Rotterdam Study.[72]

I used the same approach as Simon et al., with two key differences: first, the population comprised individuals who were free of parkinsonism and dementia at baseline (1990 or 2000), and second, I followed them up for the onset of newly-diagnosed parkinsonism, dementia or death until January 1, 2013. I genotyped 10,518 individuals for three single nucleotide polymorphisms (SNPs) of interest in the *SLC2A9* gene (rs6855911;rs7442295;rs16890979), which explains most of the genetically specified variance in serum urate.[69] Analyses were adjusted for study subcohort, sex, age, and use of diuretics. At baseline, individuals with $\geq$3 *SLC2A9* risk alleles had lower serum urate concentrations than individuals with $\leq$2 risk alleles (two-tailed p value<0.001). After a median of 11.1 year of follow-up, I observed an inverse association of the *SLC2A9* risk score with incident PD (hazard ratio= 1.10, 95% confidence interval [1.00; 1.21]; p=0.046). This effect was not modified by sex (p for interaction term of *SLC2A9* risk score and sex =0.84) or age (p for interaction term=0.78).

There is a need for replication of these results in other studies, especially given the relatively wide confidence intervals. Still, I believe that these observations provide preliminary support to the hypothesis that urate has causal neuroprotective effects related to PD, adding that these effects may start years before clinical PD diagnosis. Urate-elevating interventions were recently shown to be safe and tolerable in newly diagnosed PD patients.[73] If confirmed by similar studies, our findings open the door to urate-elevating intervention trials in individuals with probable prodromal PD.

PREDICTION OF PD AND OTHER NEURODEGENERATIVE DISEASES

Clinical context

The advanced stage of pathology at clinical diagnosis of PD likely contributes to the failure of trials that aim to slow disease progression.[74] Therefore, our battle against PD critically depends on the development of interventions that modify disease progression in individuals with prediagnostic PD, ideally preventing onset of clinical PD altogether. To identify prediagnostic PD patients, or individuals who are at high risk of clinical PD, accurate risk stratification methods are required. There is currently no evidence to suggest that a single predictor could discriminate future clinical PD patients from others; therefore, integration of predictors is necessary. Currently, only a few studies have been published that assess multivariable prediction models for PD,[75-78] none of which was shown to have high predictive utility for clinical PD over longer intervals. Three groups of predictors warrant attention: predisposing factors, biomarkers, and prodromal features.

As for predisposing factors, the identification of genetic and environmental factors that are associated with the risk of PD has fueled the idea that prediction of PD, based on these predisposing factors, might be possible in individuals who are (still) asymptomatic. In **Chapters 5.1 and 6.1**, I showed that, in isolation, risk scores based on genetic or midlife environmental factors (e.g., smoking, use of cardiovascular medication) currently do not allow for accurate PD risk prediction in the community. The results in **Chapter 6.1** suggest that combining data on genetic and midlife environmental risk markers yields a small improvement in the accuracy of PD risk prediction beyond traditional risk markers, although predictive measures were still moderate at best (i.e., C-statistic~0.7). Therefore, identification

of additional genetic and non-genetic predisposing factors, as well as their combined effects, remains a priority.

In recent years, several putative biomarkers of relatively advanced prediagnostic progression of PD pathology have been proposed, including imaging (e.g., striatal dopamine transporters uptake, substantia nigra echogenicity), cerebrospinal fluid (e.g., α-synuclein), blood (e.g., glucocerebrosidase activity) and skin (e.g., α-synuclein) biomarkers.[36,37,79-81] Over short intervals, these biomarkers may be useful for the prediction of PD, especially in selected samples of high-risk individuals. To date, however, the predictive utility over longer intervals of these biomarkers remains uncertain.

As a consequence of evolving pathology, individuals who are in the prediagnostic phase of PD often experience a combination of prodromal motor and non-motor signs and symptoms that affect their daily activities (**Chapter 7.1**). Features of prodromal PD include both subtle motor deficits as well as non-motor deficits such as RBD, autonomic dysfunction, depression[82] cognitive dysfunction (**Chapter 7.2**) and sleep complaints (**Chapter 7.3**). Interestingly, occurrence of these features can lead to falls[83] and may even influence the professional occupation of prediagnostic PD patients (**Chapter 6.3**). However, the predictive utility of non-motor features is highest in the last few years before clinical diagnosis, while evidence for any predictive value of these features over longer intervals is scarce, with exception of RBD and cognitive dysfunction (**Chapter 7.2**), which may each contribute to the identification of high-risk subgroups.

Taken together, it is currently not possible to identify the majority of prediagnostic PD patient in the general population. However, several groups of putative predictors may facilitate clinical prediction of PD in the future.

Methodological considerations on how to move forward

Four methodological issues warrant careful consideration in prediction studies of PD: the target group, setting, assessment conditions, and outcome.

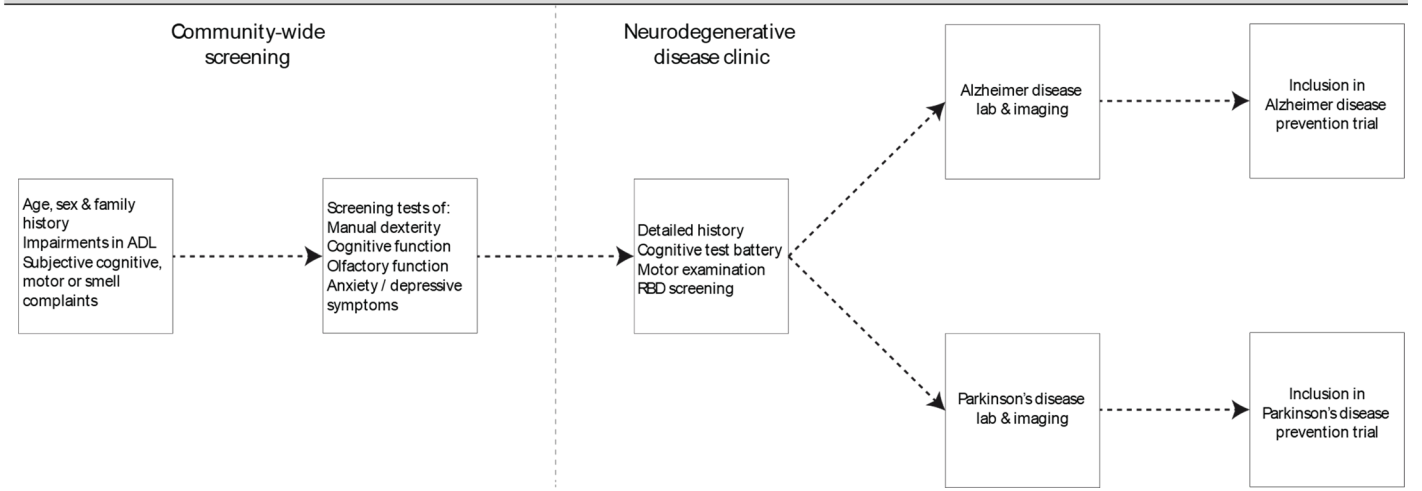
As for the target group, one approach to predict PD is to assesses prodromal features and markers of advanced pathological evolution. The first MDS criteria for prodromal PD revolve to a large extent around such markers, and I identified several additional putative predictors that fall into this category, including subtle deficits in daily functioning (**Chapter 3.3.1**), cognitive functioning (**Chapter 3.2.2**) and sleep (**Chapter 3.3.3**). For short follow-up periods, such prediction algorithms may have high predictive accuracy. However, the models would especially identify individuals who are close to clinical diagnosis. This suggests that the potential delay in disease progression that could be achieved in putative disease-modifying trials by including such individuals, instead of newly diagnosed clinical PD patients, is relatively limited. Of note, RBD may be the exception, as it is fairly specific (albeit not pathognomic[84]) for α-synucleinopathy and may precede clinical PD by decades. An alternative approach to predict PD is to target individuals at risk who are (still) asymptomatic, as putative disease-modifying treatments would potentially have the largest effects in these individuals. Such prediction models would typically include genetic markers and midlife environmental risk factors. Our data in **Chapter 6.1** emphasize the promise of combining data on genetic and environmental risk markers, and the stark difference in predictive accuracy in males versus females suggests that prediction of PD based on currently known risk markers may vary substantially across subgroups in the population. In particular, it is currently largely unknown to what extent accuracy of prediction of PD based on non-genetic data varies across life-periods, genetic predisposition, or smoking status. In the coming years,

collaboration across prospective cohort studies will facilitate examination of composite effects of potentially modifiable factors on the risk of PD in the whole population as well as in specific genetic and non-genetic subgroups.

As for the setting of prediction studies, it is important to consider the feasibility of assessment of potential predictors. With the advance of automated quantification methods and the decrease of costs, current limitations to the feasibility of some assessments, such as genetic markers or quantitative motor modalities, may decrease over time. Still, the burdensome and time-consuming nature of other assessments, such as video-polysomnography with EMG (which is the diagnostic gold standard for RBD), will likely preclude their applicability on a population-wide scale. An alternative, population-feasible approach to predict PD may involve step-wise algorithms in which a large group of individuals (e.g., all $\geq$60 year old individuals in a community) is subjected to screening tests and only screen-positive individuals are referred to a movement disorder clinic for refined risk stratification. In fact, such algorithms could be expanded to other common neurodegenerative diseases such as Alzheimer's Disease, which share several prediagnostic features with PD. In *Box 7*, I present a simplified theoretical framework to illustrate how such algorithms could be used to identify individuals at high risk of different neurodegenerative diseases.

As for conditions, it is important to note that I only assessed motor functioning under single-task conditions in this thesis. By contrast, dual-task assessments require participants to simultaneously perform a motor and cognitive test.[85]

Box 7. Step-wise screening for neurodegenerative disease trials.



ADL, activities of daily living. RBD, REM Behavior sleep Disorder.

An important question is whether dual-task assessments may have incremental predictive utility for neurodegenerative diseases. In this context, recent evidence on associations between gait and incident dementia in patients with mild cognitive impairment provides some helpful clues. In particular, associations of gait with incident dementia in these patients are amplified if gait is assessed under dual-task conditions. It remains unclear whether dual-task assessments also have incremental predictive value in adults who do not have overt cognitive dysfunction (yet). If so, dual-task gait assessments might contribute to the selection of high-risk individuals for putative neuroprotective trials aimed at delaying clinical onset of dementia. In this context, a particularly promising gait aspect is variability (e.g., high variability in stride length and time). Even under single-task conditions, variability is associated with cognitive functioning and incident dementia independently of gait speed.[86,87] Under dual-task conditions, gait variability changes disproportionally more than gait speed does, and that difference is more distinct among MCI patients than among cognitively unimpaired individuals.[88] Furthermore, it remains unclear to what extent the 'dual-task cost' varies by the complexity of the specific cognitive task administered. In fact, it is plausible that the task-specificity of 'dual-task cost' could vary by cognitive functioning, as complex dual-tasks may be better at identifying high-risk individuals for dementia in cognitively asymptomatic populations than in MCI patient populations. Of note, it is unclear to what extent findings can be generalized to neurodegenerative diseases that are primarily characterized by parkinsonism, such as PD.

Aside from predicting onset of clinical PD, there is currently huge interest in predicting the occurrence of specific features in patients with PD, in particular cognitive dysfunction, as such patients could be included in putative disease-

modifying intervention trials. The recent identification of markers of cognitive decline mediated by synucleinopathy, such as α-synuclein in skin-biopsy(31)) or coexisting pathology (e.g., Aβ42 on PET-imaging(35) has fueled the notion that etiology-based markers may improve prediction of cognitive dysfunction in PD beyond conventional markers. In fact, a recent meta-analysis of observational clinical PD cohort studies suggested that inclusion of genetic data as well as a range of phenotypical markers improved prediction of cognitive dysfunction in PD. When looking at the selected predictors, however, it is noteworthy that age alone contributed 56·5% to the model's accuracy, followed by baseline MMSE (7·7%) and five other predictors (explained variance of each <5·5%). Cognitive decline typically shows long-term tracking within individuals,[89] and in PD patients cognition typically follows a gradual downward trajectory over the course of several years (**Chapter 7.1**). By definition, this ensures that baseline cognitive functioning is a strong predictor of future decline. Furthermore, age and MMSE are by no means specific to cognitive dysfunction in PD, as they predict cognitive dysfunction in individuals without PD as well. Conversely, PD patients with a normal MMSE score can still have a range of cognitive deficits.[90] Inclusion of cognitive tests more sensitive to typical cognitive deficits in PD (e.g., visuospatial deficits) would likely have substantially improved the model's predictive accuracy, further diminishing any residual utility of other predictors besides age and baseline cognitive functioning to the model. It is therefore questionable if in the long run this prediction model will prove useful beyond using age and baseline cognition, which is already standard clinical management with respect to assessing a patient's risk of cognitive dysfunction. As of now, therefore, prediction of cognitive dysfunction in PD remains almost exclusively based on age and current cognitive functioning.

CONCLUSION

In this chapter, I have highlighted how observations from the general population have added to the understanding of motor functioning and the prediagnostic phase of PD, and I have provided my perspective on how the main findings of my thesis fit into the larger context of etiologic and predictive research of motor functioning and PD. I expect that in the coming years, implementation of quantitative motor function modalities on a broad scale will increase the accuracy of motor functioning assessments, which will facilitate studies aiming to unravel determinants and underlying mechanisms of motor functioning and PD. In turn, such studies which will accelerate the shift towards pathological mechanism-based classification of PD patients and possibly enable targeted selection of prediagnostic PD patients for putative disease-modifying intervention trials. Ultimately, such interventions may lead to a lower lifetime risk of PD. Given these prospects, I willfully choose to ignore the irony of ending this section with a two-century old quote by sharing the optimism expressed by James Parkinson:

There appears to be sufficient reason for hoping that some remedial process may ere long be discovered, by which, at least, the progress of the disease may be stopped.

James Parkinson. An essay on the shaking palsy; p. 12. London: Sherwood, Neely and Jones; 1817.

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Chapter 9

Summary

SUMMARY (English)

Context, rationale and overall approach

In **Chapter 1**, I explain the context of the research presented in this thesis, outline key gaps in knowledge on motor functioning and Parkinson's Disease (PD), and present an overall approach to address these gaps. The overall aim of this thesis is to obtain novel insight on determinants of motor functioning and the prediagnostic phase of PD.

Motor functioning

In **Chapters 2 and 3**, I assessed upper and lower extremity motor functioning using quantitative assessments, which facilitate higher accuracy of motor function assessment than qualitative assessments. Application of these modalities contributed to the identification of genetic (**Chapter 2.1**), cerebral microstructural (**Chapter 2.2**) and metabolic (**Chapter 2.3**) determinants of motor functioning. In particular, I observed that genetic variants implicated in PD affect gait in individuals with below-average cognitive functioning, and also affect cognitive functioning in individuals with below-average gait (**Chapter 2.1**). I also identified cerebral microstructural determinants of motor functions (**Chapter 2.2**). Furthermore, I uncovered a metabolic determinant of motor functioning, as subclinical variation in renal function is associated with gait, especially with shuffling gait, which is a hallmark of (prediagnostic) PD (**Chapter 2.3**).

The use of quantitative motor function assessment methods also contributed to the uncovering of a potential predictive role of motor functioning for cognitive decline and dementia (**Chapters 3**). In **Chapter 3.2**, I showed that poor manual

dexterity is not only associated with incident parkinsonism, but also with incident dementia. In fact, aside from manual dexterity, other motor functions may also signal ongoing prediagnostic pathology in individuals who are destined to develop dementia. I showed that poor gait precedes cognitive decline and incident dementia (**Chapter 3.3**) and that the combination of gait and cognitive complaints may have incremental predictive utility for incident dementia (**Chapter 3.4**).

Parkinson's Disease

In **Chapter 3.1**, I showed that parkinsonism (including PD), dementia and stroke commonly overlap in the general population, and that the overall risk of such a common neurological disease is almost one in two for women and one in three for men. In **Chapters 4 through 7**, I zoomed in on temporal trends (**Chapter 4**), genetic (**Chapter 5**) and non-genetic predisposition (**Chapter 6**), and prodromal changes (**Chapter 7**) of PD.

In **Chapter 4.1**, I reported that the incidence of PD declined over time. Interestingly, however, mortality associated with PD rose over time (**Chapter 4.2**). These seemingly paradoxical results highlight the need for insight into causal mechanisms that drive differential trends and facilitate prediction of PD, which I sought to obtain in the next two chapters. In **Chapter 5.1, I** uncovered effects of genetic variants implicated in PD on daily functioning in individuals who were (still) free of clinical PD, and also showed that these variants currently do not improve prediction of PD beyond traditional markers. In **Chapter 5.2, I** applied an alternative approach to the identification of genetic variants implicated in PD by restricting a genetic discovery study to miRNAs-related variants, which are

involved in the regulation of gene expression, and I identified 34 miRNA-related variants that are associated with PD.

In **Chapter 6.1**, I showed that previously identified early- or midlife environmental factors currently do not allow for accurate PD risk prediction in the community, however, that the combination of these factors and genetic factors did slightly improve prediction of PD beyond age and sex. In **Chapter 6.2**, I showed that large-vessel disease was not associated with incident parkinsonism. Interestingly, I observed that professional occupations are associated with incident PD in **Chapter 6.3**, suggesting that dopaminergic degeneration affects choice of occupation, which may already start in the prediagnostic phase of PD. In fact, individuals who are in the prediagnostic phase of PD often experience a combination of early motor and non-motor signs and symptoms that affect their daily activities, as I observed in **Chapter 7.1**. Furthermore, I detected cognitive dysfunction as a prodromal feature of PD in **Chapter 7.2**. Interestingly, individuals who are in the prediagnostic phase of parkinsonism also commonly report sleep problems **(Chapter 7.3)**.

Integration of key findings

In **Chapter 8**, I discuss the main findings of this thesis in a broader clinical context, point out methodological considerations that merit attention in the interpretation of these findings, and offer directions for future research.

Chapter 9

SAMENVATTING (NEDERLANDS)

Context, motivatie en algehele aanpak

In **Hoofdstuk 1** bespreek ik de context van het onderzoek dat ik in dit proefschrift presenteer, belicht ik hiaten in kennis over het motor functioneren en de Ziekte van Parkinson (ZvP) en presenteer ik mijn algehele aanpak om deze hiaten te addresseren. Het hoofddoel van dit proefschrift is om nieuw inzicht te vergaren in de determinanten van het motor functioneren en de prediagnostische fase van de ZvP.

Het motor functioneren

In **Hoofdstukken 2 en 3** heb ik het motor functioneren van de bovenste en onderste extremiteiten bestudeerd middels kwantitatieve methoden, waarmee het mogelijk is om het motor functioneren accurater te beoordelen dan met kwalitatieve methoden. Mede door toepassing van deze methoden kon ik genetische (**Hoofdstuk 2.1**), cerebrale microstructurele (**Hoofdstuk 2.2**) en metabole (**Hoofdstuk 2.3**) determinanten van het motor functioneren identificeren. Ik observeerde met name dat genetische varianten die betrokken zijn bij de ZvP invloed hebben op het looppatroon van individuen die cognitief matig functioneren, en ook invloed hebben op het cognitief functioneren van individuen die een matig looppatroon hebben (**Hoofdstuk 2.1**). Daarnaast identificeerde ik cerebrale microstructurele determinanten van verschillende motor functies (**Hoofdstuk 2.2**). Verder ontrafelde ik een associatie tussen subklinische variatie in de nierfunctie en het looppatroon, in het bijzonder een

associatie met schuifelen, wat een kenmerk is van de (prediagnostische) ZvP (**Hoofdstuk 2.3**).

De toepassing van kwantitatieve onderzoeken droeg ook bij aan het ontdekken van een mogelijke voorspellende waarde van het motor functioneren voor het optreden van cognitief verval en dementie (**Hoofdstuk 3**). In **Hoofdstuk 3.2** liet ik zien dat matige handvaardigheid niet alleen geassocieerd is met het optreden van parkinsonisme, maar ook met het optreden van dementie. Ook subtiele beperkingen in andere motor functies dan handvaardigheid kunnen een reflectie zijn van prediagnostiche pathologie in individuen bij wie later dementie gediagnosticeerd wordt. Zo toonde ik in **Hoofdstuk 3.3** aan dat een matig looppatroon een voorloper kan zijn van cognitief verval en incidente dementie, en liet ik in **Hoofdstuk 3.4** zien dat de combinatie van een matig looppatroon en cognitieve klachten mogelijk een voorspellende waarde voor dementie heeft (**Hoofdstuk 3.4**).

Ziekte van Parkinson

In **Hoofdstuk 3.1** toonde ik aan dat het optreden van parkinsonisme (inclusief de ZvP), dementie en een beroerte vaak overlappen, en dat het levensrisico van deze neurologische aandoeningen in de algemene bevolking bijna 1 op 2 is voor vrouwen en 1 op 3 voor mannen. In **Hoofdstukken 4 tot en met 7** ging ik dieper in op verschuivingen in de tijd van (**Hoofdstuk 4**), genetische (**Hoofdstuk 5**) en niet-genetische predispositie voor (**Hoofdstuk 6**), en prodromale veranderingen (**Hoofdstuk 7**) in de ZvP.

In **Hoofdstuk 4** liet ik zien dat de incidentie van de ZvP in de afgelopen decennia gedaald is (**Hoofdstuk 4.1**, terwijl de sterfte geassocieerd met de ZvP opmerkelijk genoeg juist over de tijd steeg (**Hoofdstuk 4.2**). Deze ogenschijnlijk tegenstrijdige bevindingen onderstrepen hoe belangrijk het is om nieuw inzicht te verkrijgen in determinanten van de ZvP die verschuivingen over de tijd kunnen veroorzaken. Het identificeren van zulke determinanten staat centraal in de volgende twee hoofdstukken. In **Hoofdstuk 5.1** ontdekte ik effecten van genetische varianten die betrokken zijn bij de ZvP op het dagelijks functioneren van individuen die de klinische diagnose ZvP (nog) niet hadden, en toonde ook aan dat deze varianten momenteel predictie van de ZvP niet verbeteren ten opzichte van traditionele risicoindicatoren. In **Hoofdstuk 5.2** identificeerde ik 34 genetische varianten die geassociateerd zijn met de ZvP door een genetische associatiestudie te beperken tot miRNAs-gerelateerde varianten, die betrokken zijn bij de regulatie van gen expressie.

In **Hoofdstuk 6.1** liet ik zien dat een accurate voorspelling van het optreden van de ZvP in de algemene bevolking momenteel niet mogelijk is als er alleen gebruik wordt gemaakt van eerder ontdekte omgevingsfactoren, hoewel een combinatie van deze factoren en genetische factoren de voorspelling van het optreden van de ZvP enigszins verbetert ten opzichte van leeftijd en geslacht. In **Hoofdstuk 6.2** toonde ik aan dat atherosclerose niet geassocieerd Is met het optreden van parkinsonisme. Opmerkelijk genoeg observeerde ik in **Hoofdstuk 6.3** dat bepaalde beroepen geassocieerd zijn met het optreden van van de ZvP, wat suggereert dat dopaminerge degeneratie de beroepskeuze kan beïnvloeden, mogelijk zelfs al in de prediagnostische fase van de ZvP. De prediagnostische fase van de ZvP staat verder in de schijnwerpers in het volgende hoofdtuk. In **Hoofdstuk 7.1** toonde ik aan dat individuen die zich in deze fase bevinden vaak

een combinatie van vroege motore en niet-motore kenmerken hebben die hun dagelijks functioneren beïnvloeden. Daarnaast ontdekte ik in **Hoofdstuk 7.2** dat cognitieve dysfunctie een prediagnostisch kenmerk van de ZvP kan zijn. Ook nam ik waar dat individuen die zich in deze fase bevinden vaak slaapproblemen rapporteren **(Hoofdstuk 7.3)**.

Integratie van hoofdbevindingen

In **Hoofdstuk 8** bespreek ik de belangrijkste bevindingen in dit proefschrift in een bredere klinische context, belichtte ik methodologische overwegingen die aandacht verdienen in de interpretatie van deze bevindingen, en geef ik suggesties voor verder onderzoek.

Chapter 10

Acknowledgments

Reflecting on the work that ultimately resulted in this thesis, I realize how misleading it is that mine is the only name listed on the cover of the book. Many colleagues, friends and relatives have contributed to this thesis, and I am sincerely grateful for their efforts. It is simply not possible to acknowledge all those individuals here, however, I feel obligated to at least mention a few groups of individuals who helped shape the research presented in this thesis:

My promotors Prof.dr. Ikram and Prof.dr. Koudstaal:

Arfan, from our first conversation onwards, I knew I was not interested in offers for PhD positions from other senior researchers. Our meetings -although rarely as brief as I would anticipate- were a true highlight. Irrespective of whether our discussions concerned scientific content, career opportunities, sports, or other topics, your analytical skills, ability to compartmentalize and willingness to think outside the box would always shine through. Despite our limited geographical overlap, you always seemed close by and readily available to add value to the work that has resulted in this thesis.

Peter, thank you for your contributions to the case ascertainment process as well as to various manuscripts. I have always appreciated your approachability towards me, even when I was a Medical student. Furthermore, your efficiency and attention to detail are admirable, and I can honestly say that your passion for clinical Neurology has rubbed off on me.

My copromotor Dr. Ikram:

Kamran, I am grateful for your input on several projects and also for your valuable career advice. I enjoyed sharing an office with you -as well as many meals at a certain restaurant- in Boston.

Members of my committee:

Prof.dr. Bonifati (Secretary; reading committee), Prof.dr. Bloem (reading committee), Dr. Dufouil (reading committee), Dr. Boon, Prof.dr. Oertel and Prof.dr. Ribbers. The prospect of defending this thesis in face of leading experts in such diverse fields of research is both humbling and motivating to me. Thank you for being part of my committee.

Prof.dr. Hofman:

Bert, your influence on the origins of this thesis is obvious on many levels. You accepted my application to be enrolled in a research master program in Health Sciences parallel to my medical studies, turned my focus to Epidemiology in a rousing course on the fundamentals of the field, and offered me the opportunity to work in your group at the Harvard T.H. Chan School of Public Health. You also founded the Rotterdam Study, which provided me with high-quality data to address the aims of this thesis. I consider it a true privilege to have worked closely with you and count myself among the generations of Epidemiologists you have inspired across the world.

Participants and staff of the Rotterdam Study:

The altruism of thousands of middle-aged and elderly inhabitants of Ommoord has made it possible to obtain insight on the earliest phases of a range of diseases, including Parkinson's Disease. As the study approaches its thirtieth anniversary, their willingness to commit to the Rotterdam Study is astonishing. In addition to the study's participants, I would like to express my gratitude to the dedication of the staff at the Rotterdam Study center and the Department of Epidemiology. In particular, I would like to highlight Frank, Jolande, Marlies and Nano. You played a key role in facilitating the collection, management or analysis of data that I used to address the aims of this thesis.

Key early influences on my research:

Between July 2010 and April 2015, I combined my medical studies with a research master program in Health Sciences. During that period, I learned about the fundamentals of medical research in general, and Epidemiology in particular. I would like to express my gratitude to several individuals who played a key role in that process:

Prof.dr. Frens: Maarten, thank you for bringing me in touch with fellow, highly motivated students in the Erasmus MC Honours Class, some of whom would evolve into close colleagues and friends of mine. *Prof.dr. Stricker:* Bruno, thank you for giving me the opportunity to apply epidemiological principles in practice by supervising my first research paper and playing a vital role in directing my research focus to Parkinson's Disease Epidemiology. *Prof.dr. Oscar Franco* and *Prof.dr. Pieter van Doorn:* thank you for supporting my early steps as an independent researcher. *Maarten Leening:* I am grateful for your valuable mentorship during my research master period.

Chapter 10

Key influences on my research during my PhD period:

Most of the studies in this thesis were performed during my fulltime research period, which I spent at Erasmus MC in Rotterdam (April 2015 – March 2016) and at the Harvard T.H. Chan School of Public Health in Boston (April 2016 – April 2017). Furthermore, I used my post-fulltime research period (May 2017 – May 2019) to complete ongoing studies and to build on previous findings by conducting several novel studies. That period was spent in Nijmegen, as it coincided with the first two years of my clinical residency in Neurology at Radboudumc. During each of these periods, I was lucky to be surrounded by colleagues who substantially contributed to the work that resulted in this thesis:

Frank: what a fortune it is that our PhD periods overlapped. Despite the remarkable similarities in our personal priorities, our scientific input was in many ways complimentary, which contributed substantially to the quality of this thesis. Special thanks also to *Daniel*.

Layal: you might be my favorite brilliant, outspoken and warm person to argue with. It is still difficult to believe that we are not related.

My (other) office mates Lotte, Rens, Reem and Jana: thank you for exchanging innumerous ideas with me, keeping up with my (overly) energetic presence, and maintaining a pleasant attitude throughout.

My (other) co-authors: opposing views among (co-)authors often improve the clarity of arguments concerning the rationale, design, analysis and overall interpretation of a study. I feel grateful to have worked with highly talented and motivated colleagues during my PhD period who often brought out the best in me. I would like to highlight *Vincent* and *Silvan*: thank you for collaborating with me so effectively and pleasantly on multiple projects.

My fellow Principal Investigators of the Erasmus Foosball Cohort: thank you for intensively collaborating with me on pilot studies; not finishing the subsequent cohort study is probably the biggest regret of my fulltime research period.

*My colleagues in Nijmegen, both at Radboudumc and at CWZ: t*hank you for welcoming me so warmly on a personal level and broadening my clinical and methodological horizon on a professional level. In particular, I would like to thank *Prof.dr. Bloem:* Bas, your combination of in-depth knowledge on movement disorders, creativity and ability to get things done is truly unique. I have been inspired by the start of our collaboration and look forward to working closely with you, *Marten, Jan,*

Angelika, Marjan, Nienke and our other colleagues at the Parkinson Center Nijmegen in the coming years.

Other pleasant distractors in-between research hours: aside from those who directly contributed to research output, I am grateful to several close friends for providing me with pleasant distraction from and (sometimes unintended) ideas for research: *Maxim, Michiel, Rick, Zjiwar* and the *Wolf Pack*. Special thanks also to various colleagues-turned-friends, including the *Poli-musketeers* and *Intervisionaries (Jildou, Mijntje & Nina)* as well as other members of *De Harde Kern, HSPH Lunch Team, Netherlands Boston Alliance,* and *Strong Men Moving Team*.

My paranymphs, whom I am incredibly proud to have by my side during the defense:

Rooj, my brother. I can hardly think of a better illustration of multiplicative interaction on personal development than us growing up together. Before, during and after the ceremony, you and I will always remain on the same side… except when it comes to your inexplicable love for Ajax. *Hieab*, my dear friend. Aside from your undeniable brilliance, you are a true symbol of loyalty and generosity. You have had a major influence on me at key junctures in my career. I look forward to seeing you and Rooj team up during the defense.

My family:

Thank you for the joy and inspiration you have always brought me, including during the development of this thesis. I am grateful that many of my relatives, including Marouschka, will be able to attend the defense ceremony, and I cherish their support. I would also like to acknowledge the inspirational influence of my late grandparents (Opa&Oma, Djiddu&Djidde) and auntie Fawzia, which remains vivid in my thoughts.

To my parents and stepmother: I am grateful for your unconditional support and love. Dineke, thank you for always being there for Rooj and me. Mom, your unrelenting curiosity and in-depth knowledge on a broad array of topics are exceptional and make your influence on the origins of this thesis obvious. In a broader perspective, I am extremely grateful to you and dad for providing me with the opportunities that paved the way for this thesis. Thank you both also for your contributions to my prepositions. Dad, you are my role model, my guide and my friend. Thank you.

Chapter 11

Appendix

Chapter 11

LIST OF PUBLICATIONS AND MANUSCRIPTS

1. Akoudad S, **Darweesh SKL**, Leening MJ, Koudstaal PJ, Hofman A, van der Lugt A, Stricker BH, Ikram MA, Vernooij MW. Use of coumarin anticoagulants and cerebral microbleeds in the general population. Stroke. 2014;45:3436-9.
2. Baena CP, Olandoski M, Younge JO, Buitrago-Lopez A, **Darweesh SKL**, Campos N, Sedaghat S, Sajjad A, van Herpt TT, Freak-Poli R, van den Hooven E, Felix JF, Faria-Neto JR, Chowdhury R, Franco OH. Effects of lifestyle-related interventions on blood pressure in low and middle-income countries: systematic review and meta-analysis. Journal of hypertension. 2014;32:961-73.
3. Bano A, Chaker L, **Darweesh SKL**, Korevaar TI, Mattace-Raso FU, Dehghan A, Franco OH, van der Geest JN, Ikram MA, Peeters RP. Gait patterns associated with thyroid function: The Rotterdam Study. Scientific reports. 2016;6:38912.
4. Bloem BR, Eimers M, Van Galen M, Munneke M, **Darweesh SKL**. From trials to clinical practice: Lessons from a Dutch implementation program for Parkinson patients. Submitted.
5. Bos D, Wolters FJ, **Darweesh SKL**, Vernooij MW, de Wolf F, Ikram MA, Hofman A. Cerebral small vessel disease and the risk of dementia: A systematic review and meta-analysis of population-based evidence. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2018;14:1482-92.
6. Chibnik LB, Wolters FJ, Backman K, Beiser A, Berr C, Bis JC, Boerwinkle E, Bos D, Brayne C, Dartigues JF, **Darweesh SKL**, Debette S, Davis-Plourde KL, Dufouil C, Fornage M, Grasset L, Gudnason V, Hadjichrysanthou C, Helmer C, Ikram MA, Ikram MK, Kern S, Kuller LH, Launer L, Lopez OL, Matthews F, Meirelles O, Mosley T, Ower A, Psaty BM, Satizabal CL, Seshadri S, Skoog I, Stephan BCM, Tzourio C, Waziry R, Wong MM, Zettergren A, Hofman A. Trends in the incidence of dementia: design and methods in the Alzheimer Cohorts Consortium. European journal of epidemiology. 2017;32:931-8.
7. **Darweesh SKL**, Adams HHH, Van der Geest JN, Ikram MK, Ikram MA. Parkinson Disease genes, gait and cognition. Submitted.
8. **Darweesh SKL**, Ikram MK, Faber MJ, de Vries NM, Haaxma CA, Hofman A, Koudstaal PJ, Bloem BR, Ikram MA. Professional occupation and the risk of Parkinson's disease. Eur J Neurol. 2018;25:1470-6.
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10. **Darweesh SKL**, Koudstaal PJ, Ikram MK, Ikram MA. Cognitive decline before diagnosis of Parkinson's disease. The Lancet Neurology. 2017;16:262.
11. **Darweesh SKL**, Koudstaal PJ, Stricker BH, Hofman A, Ikram MA. Trends in the Incidence of Parkinson Disease in the General Population: The Rotterdam Study. American journal of epidemiology. 2016;183:1018-26.

12. **Darweesh SKL**, Koudstaal PJ, Stricker BH, Hofman A, Steyerberg EW, Ikram MA. Predicting Parkinson disease in the community using a nonmotor risk score. European journal of epidemiology. 2016;31:679-84.
13. **Darweesh SKL**, Leening MJ, Akoudad S, Loth DW, Hofman A, Ikram MA, Vernooij MW, Stricker BH. Clopidogrel use is associated with an increased prevalence of cerebral microbleeds in a stroke-free population: the Rotterdam study. Journal of the American Heart Association. 2013;2:e000359.
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15. **Darweesh SKL**, Polinder S, Mulder MJ, Baena CP, van Leeuwen N, Franco OH, Jacobs BC, van Doorn PA. Health-related quality of life in Guillain-Barre syndrome patients: a systematic review. Journal of the peripheral nervous system : JPNS. 2014;19:24-35.
16. **Darweesh SKL**, Raphael KG, Brundin P, Matthews H, Wyse RK, Chen H, Bloem BR. Parkinson Matters. Journal of Parkinson's disease. 2018;8:495-8.
17. **Darweesh SKL**, Roshchupkin GV, Licher S, Van der Geest JN, Ikram MK, Ikram MA. Microstructural brain determinants of motor functioning. In preparation.
18. **Darweesh SKL***, Verlinden VJ*, Adams HH*, Uitterlinden AG, Hofman A, Stricker BH, van Duijn CM, Koudstaal PJ, Ikram MA. Genetic risk of Parkinson's disease in the general population. Parkinsonism & related disorders. 2016;29:54-9.
19. **Darweesh SKL***, Verlinden VJ*, Stricker BH, Hofman A, Koudstaal PJ, Ikram MA. Trajectories of prediagnostic functioning in Parkinson's disease. Brain. 2017;140:429-41.
20. **Darweesh SKL**, Verlinden VJ, Ikram MA. Dual-Task Gait and Incident Dementia: A Step Forward, but Not There Yet. JAMA neurology. 2017;74:1380.
21. **Darweesh SKL**, Wolters FJ, Cremers LG, Hofman A, Koudstaal PJ, Vernooij MW, Ikram MK, Bos D, Vernooij MW, Ikram MA. Motoric cognitive risk syndrome: risk factors, MRI determinants, and prognosis in a community-based cohort. In preparation.
22. **Darweesh SKL**, Wolters FJ, Hofman A, Stricker BH, Koudstaal PJ, Ikram MA. Simple Test of Manual Dexterity Can Help to Identify Persons at High Risk for Neurodegenerative Diseases in the Community. The journals of gerontology Series A, Biological sciences and medical sciences. 2017;72:75-81.
23. **Darweesh SKL**, Wolters FJ, Ikram MA, Bos D, Hofman A. Broadening the scope of epidemiologic dementia research. European journal of epidemiology. 2018;33:617-20.
24. **Darweesh SKL**, Wolters FJ, Ikram MA, de Wolf F, Bos D, Hofman A. Inflammatory markers and the risk of dementia and Alzheimer's disease: A meta-analysis. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2018;14:1450-9.
25. **Darweesh SKL**, Wolters FJ, Postuma RB, Stricker BH, Hofman A, Koudstaal PJ, Ikram MK, Ikram MA. Association Between Poor Cognitive Functioning and Risk of Incident Parkinsonism: The Rotterdam Study. JAMA neurology. 2017;74:1431-8.

26. Ghanbari M, **Darweesh SKL**, de Looper HW, van Luijn MM, Hofman A, Ikram MA, Franco OH, Erkeland SJ, Dehghan A. Genetic Variants in MicroRNAs and Their Binding Sites Are Associated with the Risk of Parkinson Disease. Human mutation. 2016;37:292-300.
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28. Janssen Daalen JM, Tosserams A, Bloem BR, **Darweesh SKL**. Olfactory function and the risk of Parkinson disease: A meta-analysis. In preparation.
29. Jaspers L, Schoufour JD, Erler NS, **Darweesh SKL**, Portegies ML, Sedaghat S, Lahousse L, Brusselle GG, Stricker BH, Tiemeier H, Ikram MA, Laven JS, Franco OH, Kavousi M. Development of a Healthy Aging Score in the Population-Based Rotterdam Study: Evaluating Age and Sex Differences. Journal of the American Medical Directors Association. 2017;18:276 e1- e7.
30. Lahousse L, Vernooij MW, **Darweesh SKL**, Akoudad S, Loth DW, Joos GF, Hofman A, Stricker BH, Ikram MA, Brusselle GG. Chronic obstructive pulmonary disease and cerebral microbleeds. The Rotterdam Study. Am J Respir Crit Care Med. 2013;188:783-8.
31. Leermakers ET, **Darweesh SKL**, Baena CP, Moreira EM, Melo van Lent D, Tielemans MJ, Muka T, Vitezova A, Chowdhury R, Bramer WM, Kiefte-de Jong JC, Felix JF, Franco OH. The effects of lutein on cardiometabolic health across the life course: a systematic review and meta-analysis. Am J Clin Nutr. 2016;103:481-94.
32. Leermakers ET, Moreira EM, Kiefte-de Jong JC, **Darweesh SKL**, Visser T, Voortman T, Bautista PK, Chowdhury R, Gorman D, Bramer WM, Felix JF, Franco OH. Effects of choline on health across the life course: a systematic review. Nutrition reviews. 2015;73:500-22.
33. Licher S*, **Darweesh SKL***, Wolters FJ, Fani L, Heshmatollah A, Mutlu U, Koudstaal PJ, Heeringa J, Leening MJG, Ikram MK, Ikram MA. Lifetime risk of common neurological diseases in the elderly population. Journal of neurology, neurosurgery, and psychiatry. 2019;90:148-56.
34. Lysen TS, **Darweesh SKL**, Ikram MK, Luik AI, Ikram MA. Sleep and risk of parkinsonism and Parkinson’s disease: a population-based study. Brain. 2019.
35. Melo van Lent D, Leermakers ETM, **Darweesh SKL**, Moreira EM, Tielemans MJ, Muka T, Vitezova A, Chowdhury R, Bramer WM, Brusselle GG, Felix JF, Kiefte-de Jong JC, Franco OH. The effects of lutein on respiratory health across the life course: A systematic review. Clinical nutrition ESPEN. 2016;13:e1-e7.
36. Sedaghat S, **Darweesh SKL**, Verlinden VJ, van der Geest JN, Dehghan A, Franco OH, Hoorn EJ, Ikram MA. Kidney function, gait pattern and fall in the general population: a cohort study. Nephrol Dial Transplant. 2018;33:2165-72.
37. Silva de Lima AL, Smits T, **Darweesh SKL**, Valenti G, Baldus H, de Vries NM, Faber MJ, Bloem BR. Home-based monitoring of falls using wearable sensors in Parkinson's disease. Submitted.

38. Smedinga M, **Darweesh SKL**, Bloem BR, Post B, Richard E. Preparing for Parkinson's Disease prevention trials - Lessons Learned from Alzheimer's disease research. In preparation.
39. Tosserams A, Araujo R, Pringsheim T, Post B, **Darweesh SKL**, IntHout J, Bloem BR. Underrepresentation of women in Parkinson's disease trials. Movement disorders : official journal of the Movement Disorder Society. 2018;33:1825-6.
40. Trajanoska K, Schoufour JD, **Darweesh SKL**, Benz E, Medina-Gomez C, Alferink LJ, Lahousse L, Brusselle G, Stricker B, Darwish Murad S, Zillikens MC, Uitterlinden AG, Ikram MA, Franco OH, Rivadeneira F. Sarcopenia and Its Clinical Correlates in the General Population: The Rotterdam Study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2018;33:1209-18.
41. Vlasov V, **Darweesh SKL**, Stricker BH, Franco OH, Ikram MK, Kavousi M, Bos D, Klaver CCW, Ikram MA. Subclinical vascular disease and the risk of parkinsonism: The Rotterdam Study. Parkinsonism & related disorders. 2017;43:27-32.
42. Wolters FJ, Chibnik LB, Waziry R, Anderson R, Berr C, Beiser A, Bis JC, Blacker D, Bos D, Brayne C, Dartigues JF, **Darweesh SKL**, Davis-Plourde KL, De Wolf F, Debette S, Dufouil C, Fornage M, Goudsmit J, Grasset L, Gudnason V, Hadjichrysanthou C, Helmer C, Ikram MA, Ikram MK, Joas E, Kern S, Kuller LH, Launer L, Lopez OL, Matthews F, McRae-McKee K, Meirelles O, Mosley T, Pase MP, Psaty BM, Satizabal CL, Seshadri S, Skoog I, Stephan BCM, Wetterberg H, Wong MM, Zettergren A, Hofman A. Time trends in the incidence of dementia over three decades in Europe and the USA: The Alzheimer Cohorts Consortium. Submitted.
43. Wolters FJ, Segufa RA, **Darweesh SKL**, Bos D, Ikram MA, Sabayan B, Hofman A, Sedaghat S. Coronary heart disease, heart failure, and the risk of dementia: A systematic review and meta-analysis. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2018;14:1493-504.
44. Ypinga JHL, Henderson E, Bloem BR, Redwood S, Smink A, Munneke M, Ben-Shlomo Y, **Darweesh SKL**. Proactive and Integrated Management and Empowerment in Parkinson's Disease (PRIME Parkinson): Rationale and Study Design. In preparation.

**These authors contributed equally.*

Chapter 11

PHD PORTFOLIO

Name PhD Student	Sirwan Khalid Lefta Darweesh
Research School	Netherlands Institute for Health Sciences
Erasmus MC department	Epidemiology
Promotors	Professors M.A. Ikram & P.J. Koudstaal
Copromotor	Dr M.K. Ikram
Full-time research period	April 2015 – April 2017

PhD Training

	ECTS*	Year(s)
General courses and seminars		
Master of Science in Health Sciences	120	2011 - 2015
Scientific integrity	0.3	2015
Departmental research seminars	2.0	2015 - 2017
Journal Club seminars	0.7	2015 - 2017
International conferences		
American Academy of Neurology Annual Meeting (Vancouver, Canada)	2.0	2016
International Parkinson and Movement Disorder Society Conference (Berlin, Germany)	2.0	2016
VasCog conference (Amsterdam, the Netherlands)	1.0	2016
American Academy of Neurology Annual Meeting (Boston, USA)	2.0	2017
European Academy of Neurology Annual Meeting (the Netherlands)	1.0	2017
Biannual Alzheimer Cohorts Consortium workshop	4.0	2016 - 2017
Research Visit		
Harvard T.H. Chan School of Public Health	N/A	2016 - 2017

Teaching activities

Teaching assistance	ECTS*	Year
Principles of research in medicine and epidemiology (NIHES)	0.5	2015
The Practice of Epidemiologic Analysis (NIHES)	0.5	2015
Fundamentals of epidemiology (Harvard T.H. School of Public Health)	4.0	2016
Project supervision		
Claudio Martins (master thesis): *Kidney function and Parkinson's Disease*	3.0	2015
Vanja Vlasov (master thesis): *Subclinical vascular disease and parkinsonism*	3.0	2016

Other activities

	ECTS*	Year
Peer review	3.0	2015 - 2017

*ECTS (European Credit Transfer System) equals a workload of 28 hours

Chapter 11

ABOUT THE AUTHOR

Sirwan Khalid Lefta Darweesh was born on February 1, 1990 in Gouda, the Netherlands. He is the eldest son of a Kurdish father (Khalid Darweesh) and a German mother (Sigrid Bollwerk). Sirwan has one sibling: his younger brother Rooj. Sirwan completed his secondary education (gymnasium) at the Christelijk Lyceum Zeist and obtained his medical degree at Erasmus University Medical Center (Erasmus MC) in 2015. During his medical studies, Sirwan was selected for the Erasmus MC Honours Class. In parallel, Sirwan completed a research master program in Health Sciences in 2015. Sirwan won a Royal Netherlands Academy of Arts and Sciences Academy Assistantship for his work as a research master student.

He subsequently started a PhD research project on the determinants of motor functioning and prediagnostic Parkinson's Disease at the Department of Epidemiology at Erasmus MC, under supervision of Prof.dr. Arfan Ikram, Prof.dr. Peter Koudstaal and Dr. Kamran Ikram (co-promotor). The results of that project are presented in this thesis. Sirwan performed part of the work at the Harvard T.H. Chan School of Public Health, where he worked as a Visiting Scientist under supervision of Prof.dr. Albert Hofman. During his time as a PhD-student, Sirwan received various personal fellowships and contributed to several successful grant applications as a co-investigator.

In 2017, Sirwan started his Residency in Neurology at Radboud University Medical Center (Chair: Prof.dr. Karin Klijn), under supervision of Dr. Bart Post. Sirwan currently also works as a postdoctoral researcher at the Parkinson Center Nijmegen (Chair: Prof.dr. Bas Bloem).