# Systemic Function Impairment and Neurodegeneration in the General Population

Systemische functiestoornissen en neurodegeneratie bij de algemene bevolking

Proefschrift

**Thesis**

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By

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# Chapter 1 General Introduction

“The bank card swiped over and over again; just said the words in a flash to forget; cannot remember dishes just eaten a few hours ago; often fail to speak the names of old friends or relatives…”. The globally estimated number of dementia cases was 57.4 million in 2019, and this figure would climb up to 152.8 million in 2050 [1]. Among the elderly, dementia has become a common chronic disease. However, the general public and even family members of patients do not know much about dementia with various misconceptions. A common (mis)conception goes, ”People will naturally get confused or senile when they get old” or ”There is no cure for dementia”. If the elderly develop the above mental symptoms and abnormal behaviors, it is likely to be the early signs of dementia.

Neurodegenerative diseases, such as dementia and Parkinson’s disease are two common brain disorders with enormous burden globally [1-4], and as the population grows older, the prevalence and incidence of these diseases rise in tandem. However, the efficacy of current drugs or medications regarding reversing or slowing the progression of neurodegenerative conditions is still being debated [5, 6]. Large clinical trials on new potential drug treatments are still ongoing. Multifactorial causes of neurodegeneration have been regarded to be the primary factor undermining the efficacy of a single medicine or monotherapy [7]. Among individuals aged 45 years and older with a non-communicable disease, at least a third would develop multiple comorbid conditions [8]. It is commonly observed that dementia patients are diagnosed with multiple comorbidities, including cardiovascular diseases, diabetes, and psychosocial problems [9, 10]. A growing body of evidence has shed light on the interplay between the other organ systems and the brain, including the brain-heart axis [11], (gut)-liver-brain axis [12], and lung-brain axis [13], and not all of the above interactions have been studied in well-designed epidemiological studies.

According to the available information, there are probable mechanisms linking systemic function deterioration to neurodegenerative disorders. First, between other systemic

comorbidities and neurodegeneration, there are shared risk factors like age, obesity, smoking, physical inactivity, etc. [14-19]. As aging-related brain conditions, the accumulative effect of harmful exposure might directly affect brain health or indirectly promote brain disorders through systemic dysfunction. That is to say, systemic dysfunction predominates the downstream pathophysiology of brain aging and may partially mediate the effect of common risk factors on the neurodegenerative process. Time-dependent alterations of aging hallmarks also reflect the cumulative effect of the imbalance between avoiding hazards that speed up aging, accepting variables that promote health, and administering or adhering to treatments [20]. As a result, risky exposures like sedentary behaviors or inactive physical activity may alter systemic risk factors, which in turn may cause neurodegenerative disorders [21].

Systemic dysfunction also relates to brain structural alterations. The integrity of brain structure guarantees the normality of neural function and brain structural deterioration clinically signs the occurrence of neurodegeneration. Alzheimer's patients consistently experience cognitive impairment, including memory loss and executive dysfunction, while brain imaging studies reveal shrinkage of healthy brain tissue and enlargement of lesions [22, 23]. Subclinical brain damage [24], particularly accumulation of white matter hyperintensity (WMH) in the frontal and internal capsule, would also undermine the neural regulation of movement and further contribute to the common occurrence of Parkinsonism in older adults [25]. The homeostatic processes of cerebral metabolism depend on the maintenance of systemic function, and the presence of peripheral abnormalities, such as circulatory inflammation, vascular lesions, oxygen deficit, insulin resistance, and so forth, would invariably to some extent interfere with normal brain aging. [26, 27].

Evidence from epidemiological studies has already demonstrated the association of some systemic disorders, i.e. diabetes mellitus [28], atrial fibrillation [29], hypertension [28], heart failure [30], coronary heart disease [30], systemic microangiopathy [31], with a higher risk of cognitive

impairment or occurrence of neurodegenerative diseases. However, it remains unclear about the roles of other understudied systemic dysfunction, such as pulmonary function restriction, liver steatosis, and low bone mineral density, in these brain disorders. As age grows, the elderly are at a higher risk of structural and functional decline in multiple organs. Exposure to other risk factors, such as smoking, obesity, and nutrition deficiency, could accelerate the dysfunction and development of co-occurring comorbidities, i.e. chronic obstructive pulmonary disease, non-alcoholic liver disease, and osteoporosis. Previous studies have reported associations between the above systemic function impairment and structural brain changes [32-34]. For instance, persons with liver steatosis had a higher fractional anisotropy and lower levels of brain perfusion and cerebral blood flow [32]. And liver fibrosis was also related to brain volumetric alterations [32]. The vast majority of patients with fatty liver disease are overweight but without significant symptoms, its link with neurodegeneration remains unexplored. A better understanding of the association between systemic malfunction and brain abnormalities would allow us to develop a multi-system strategy for neurodegeneration prevention.

## OVERALL AIM AND OUTLINE OF THIS THESIS

The overarching goal of this thesis is to get a better knowledge of the relationship between systemic dysfunction and neurodegenerative disorders, notably dementia and Parkinson's disease, in middle-aged and older persons. Neurodegeneration and reduced lung function are the main topics of **Chapter 2**. I investigated the association between incident dementia and lung function impairment, as determined by respiratory testing, in **Chapter 2.1**. Cognitive function and cerebral brain lesions were assessed in **Chapter 2.2**, and I examined variations in these markers under various degrees of lung impairment. In **Chapter 3**, I quantified the relationship between incident neurodegenerative diseases including dementia and Parkinson's disease, and non-alcoholic fatty liver disease. The link between cardiac dysfunction, as

indicated by plasma cardiac biomarkers, and neurodegeneration is the main topic of **Chapter 4**. N-Terminal Pro-B-Type Natriuretic Peptide (NT-proBNP) and alterations in cognition and brain structural imaging markers were the subjects of my **chapter 4.1** research. This chapter focused on determining if high levels of NT-proBNP accelerate cognitive decline and loss of brain tissue volumes over time, as both cognitive decline and brain structural changes are two hallmarks of dementia. In **chapter 4.2**, I evaluated the association between Parkinsonism and Parkinson's disease and three cardiac biomarkers, including NT-proBNP, high-sensitivity cardiac troponin T (hs-cTnT), and creatine kinase myocardial band (CK-MB). In **Chapter 5**, the temporal relationship between bone mineral density and dementia was investigated to gain insights into how bone mineral density occurs at the prodromal phase of dementia. Lastly, the main discussion in **Chapter 6** reviews the findings of **Chapters 2-5**, discusses methodological aspects when appraising these results, and proposes implications for prevention practices and future research.

# Chapter 2 Lung Function Impairment and Neurodegeneration

# Chapter 2.1 Lung Function Impairment and the Risk of Incident Dementia: The Rotterdam Study

**Abstract**

**Background:** The etiology of dementia may partly be underpinned by impaired lung function via systemic inflammation and hypoxia.

**Objective:** To prospectively examine the association between COPD and subclinical impairments in lung function and the risk of dementia.

**Methods:** In the Rotterdam Study, we assessed the risk of incident dementia in participants with Preserved Ratio Impaired Spirometry (PRISm; $FEV_1/FVC \geq 0.7$, FEV1<80%) and in participants with COPD ($FEV_1/FVC < 0.7$) compared to those with normal spirometry (controls; $FEV_1/FVC \geq 0.7$, $FEV_1 \geq 80\%$). Hazard ratios (HRs) with 95% confidence intervals (CI) for dementia were adjusted for age, sex, education attainment, smoking status, systolic blood pressure, body mass index, triglycerides, comorbidities and Apolipoproptein E (*APOE)* genotype.

**Results:** Of 4,765 participants, 110 (2.3%) developed dementia after 3.3 years. Compared to controls, participants with PRISm, but not COPD, had an increased risk for all-type dementia (adjusted $HR_{PRISm}$ 2.70; 95% CI, 1.53–4.75; adjusted $HR_{COPD}$ 1.03; 95% CI, 0.61–1.74). These findings were primarily driven by men and smokers. Similarly, participants with FVC% predicted values in the lowest quartile compared to those in the highest quartile were at increased risk of all-type dementia (adjusted HR 2.28; 95% CI, 1.31–3.98), as well as Alzheimer's Disease (AD; adjusted HR 2.13; 95% CI, 1.13–4.02).

**Conclusions:** Participants with PRISm or a low FVC% predicted lung function were at increased risk of dementia, compared to those with normal spirometry or a higher FVC% predicted, respectively. Further research is needed to elucidate whether this association is causal and how PRISm might contribute to dementia pathogenesis.

**Keywords:** PRISm; FVC; COPD; AD; dementia

## Introduction

Dementia is characterized by poor cognitive performance interfering with activities of daily living and impaired health-related quality of life at older ages [35], with an increasing prevalence worldwide [36]. In order to mitigate the burden of dementia through postponement or prevention, and to respond adequately on such a major health problem, the identification of key modifiable risk factors is warranted and include smoking, obesity, hypertension, depression, sleep apnea, diabetes and hyperlipidemia [37]. Chronic obstructive pulmonary disease (COPD) and decreased lung volume capacity have also been associated with a greater risk of dementia and compromised cognitive ability [38]. Possible etiological links with dementia comprise systemic inflammation and hypoxia induced oxidative stress [38-40].

More recently, Preserved Ratio Impaired Spirometry (PRISm) – with a prevalence ranging from 3% to 20% in adults [41] – has emerged as a clinically relevant entity related to premature mortality [41, 42], but thus far has been largely understudied, because of a hitherto stronger focus on COPD. The term PRISm encompasses the findings of restrictive respiratory pattern with impaired spirometry (i.e. decreased $FEV_1$ or FVC) but preserved FEV1/FVC ratio [41]. People with PRISm suffer from lung function restriction but due to normal range of FEV1/FVC ratio would not be diagnosed as COPD according to the GOLD guidelines in clinical practice [41, 43]. Previous studies have suggested PRISm is a fluctuating state, serving as an intermediate phase between normal spirometry and COPD [42, 44]. However, very little is known about the clinical sequelae of PRISm, including risk of dementia.

Therefore, the aim of this study was to investigate the association of both COPD and subclinical reduced lung function, as evidenced by the presence of impaired lung volumes (PRISm), with the risk of dementia at follow-up within a prospective population-based cohort study.

## Methods

This study was conducted within the Rotterdam Study, a prospective cohort study that started in 1990, comprising almost 15,000 participants aged at least 45 years, with the aim of studying chronic diseases in the general population [45]. Every four to five years, participants underwent follow-up examinations, consisting of a home interview and various physical examinations at the research center. We used data collected between 2009 and 2014 as baseline for this study, when participants underwent spirometry at the research center. A total of 4,765 persons with interpretable spirometry and without asthma and without prevalent dementia were retained for analyses (Figure 1).

### *Standard protocol approvals, registrations and patients consents*

The study had been approved by the medical ethics committee of the Erasmus Medical Centre (Rotterdam, the Netherlands), and the review board of the Netherlands Ministry of Health, Welfare and Sports (1068889-159521-PG). Informed consent was provided by all participants.

### *Spirometry test*

Lung function was assessed via pre-bronchodilator spirometry performed by trained paramedical personnel using a Master Screen PFT Pro (Care Fusion, Netherlands) according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines [46]. Predicted forced vital capacity (FVC) and predicted forced expiratory volume in one second ($FEV_1$) values were calculated using Global Lung Initiative (GLI) reference equations taking age, sex, height and ethnicity into account [47]. Based on these values, the following subgroups were defined: COPD ($FEV_1/FVC<70\%$), PRISm ($FEV_1/FVC\geq70\%$ and $FEV_1<80\%$ predicted) and normal spirometry ($FEV_1/FVC\geq70\%$ and an $FEV_1\geq80\%$ predicted) were distinguished [41, 43]. Spirometry was conducted in accordance with the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines [46, 47], with specific preparatory instructions, e.g. with respect to smoking or other factors. In order to guarantee the reliability

and reproducibility, at least two spirometry tests were implemented on each participant, and the best reading was obtained. No specific preparatory instructions were given (e.g. related to smoking or other factors). The quartile categories of lung function parameters were derived from values in this study, which is similar to quintile subgroups in a previous study [48]. For calculation of trending hazard ratio with 10% change in lung function, lung function parameters were included in cox models after being divided by 10. Airflow limitation was confirmed by the value of a post-bronchodilator $FEV_1$/FVC below 0.7 [43].

***Dementia assessment***

Dementia assessment was conducted for participants at baseline and subsequent center visits with the Mini-Mental State Examination and the Geriatric Mental Schedule [49]. Those with a Mini-Mental State Examination score <26 or Geriatric Mental Schedule score >0 underwent further investigation along with an interview with a research physician, that contained the Cambridge Examination for Mental Disorders of the Elderly. The whole population also underwent routine cognitive assessment. Moreover, 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. If available clinical neuroimaging was used for determining dementia subtype [45]. An adjudication panel led by a consultant neurologist established the final diagnosis according to standard criteria for dementia (Diagnostic and Statistical Manual of Mental Disorder, Third Edition-Revised: DSM-III-R) and Alzheimer's disease (AD) (National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association: NINCDS–ADRDA). Follow-up until 14 December, 2017 was virtually complete (95.5% of potential person-years). Within this period, participants were followed until the date of dementia and AD diagnosis, death, loss to follow-up or 14 December, 2017, whichever came first.

***Covariates***

The following variables were considered as possible confounders, primarily based on previous literature and their role as shared causes between lung function and dementia. Demographic information included age, sex, education level (primary education, lower education, intermediate education, higher education), smoking status (never, former, current), systolic blood pressure (mmHg), body mass index (BMI, $kg/m^2$, calculated by weight [kg] divided by height [m] squared) and chronic comorbid conditions (diabetes and stroke) [45]. Blood samples were extracted for determination of levels of triglycerides and DNA at the research center. Apolipoprotein E (*APOE*) genotype was determined using a PCR in the original cohort (RS-I, starting between July, 1989 and September, 1993) and a bi-allelic TaqMan assay (rs7412 and rs429358) on labeled DNA samples in the two cohorts (RS-II-3, starting between February, 2000 and December, 2001; and RS-III-2, starting between February, 2006 and December, 2008), respectively. This study included these three sub-cohorts. *APOE*-ε4 represented carrier of one or two ε4 alleles. Participants were categorized into three groups: high genetic risk (ε2ε4, ε3ε4 or ε4ε4 genotypes), intermediate risk (ε3ε3) or low risk (ε2ε2 or ε2ε3) [50]. As the strongest genetic risk factor for dementia, APOE has additionally potent cardiovascular effects, including arteriosclerosis and cardiac function. In this regard, APOE may also impact lung function. We therefore included APOE in the models as possible confounder [48, 51]. Missing values were handled by five-times imputation using chained equation [52].

**Statistical analysis**

Baseline characteristics are described among subgroups of lung function. Data are expressed as mean ± standard deviation (SD) for normally distributed variables or as median (interquartile range [IQR]) for non-normally distributed variables.

For analyses of the association between lung function at baseline and risk of incident dementia, we used Cox proportional-hazards regression analyses. Lung function was categorized as

normal spirometry, PRISm and COPD. In addition, lung volume capacity comprised subgroups of quartiles of $FEV_1$% predicted, FVC% predicted and ratio of $FEV_1$/ FVC. Follow-up time started on the date of spirometry test at baseline and ended until diagnosis of dementia, death, lost to follow-up, or December 14, 2017. The proportional hazards assumption was checked using Schoenfeld residuals. Model 1 was adjusted for APOE category, age, sex and education level. Model 2 was additionally adjusted for smoking status, BMI, systolic blood pressure, triglyceride and comorbidity (history of stroke and diabetes mellitus). Covariates above were selected based on previous literature knowledge, clinical relevance and availability of the data. Given the relatively small number of incident cases of dementia, we also constructed a third model in which the covariates were accounted for using propensity scores. Propensity scores were employed to reduce the number of covariates through summarizing information of variables into a single score, thus avoiding any problem of overfitting the models [53, 54]. In this study, propensity scores are the predicted probabilities of PRISm and COPD and derived by fitting logistic regression models adjusting for age, sex, education level, smoking status, systolic blood pressure, BMI, triglycerides, chronic comorbid conditions (diabetes and stroke) and APOE phenotypes.

We also studied how PRISm and COPD related to the risk of mortality to gauge the possible effect of competing risk in our associations. The competing risk, such as death before occurrence of incident dementia, are considered as independent event but is neglected in conventional methods for survival analyses, thus the true observation of the event of interest could be hindered in the presence of competing risk and then distort the association we explored [55]. For unadjusted survival analyses intended to portray absolute risks, we used sub-distribution hazard models to account for competing risks to estimate cumulative incidence of dementia and all-cause death [56].

In addition, we conducted stratified analyses in women, men, non-smoking participants, smokers and participants without APOE-e4 allele and history of stroke and diabetes. These were selected as possible effect modifiers based on previous literature and biological plausibility [37, 57-61].

## Results

### Clinical and lung functional characteristics of participants

Among 4,765 participants (mean age 68.2±12.9 years, 54.9% women), 16.0% (n=763) had COPD, 6.7% (n=319) had PRISm and 77.3% (n=3683) had normal spirometry. More than twenty percent (23.0%) of the participants received higher education, and two thirds (66.1%) were current or former smokers. The participants had a median BMI of 27.0±5.1 $Kg/m^2$, systolic blood pressure of 141±29.0 mmHg and triglyceride level of 1.3±0.8 mg/dl. While 8.4% (n=396) had a history of diabetes mellitus, 1.1% of them experienced stroke before (n=53). 1199 (27.1%) participants carried *APOE*-ε4 allele (Table 1).

### Lung function and risk of incident dementia and Alzheimer's Disease

During a median of 3.3 years of follow-up, 110 participants (2.3%) developed incident dementia, of whom 89 (1.9%) developed Alzheimer's disease. Moreover, among all participants, 292 (6.1%) died due to non-dementia related causes within the follow-up period (Table 2).

First, we evaluated the association between lung function impairment at baseline and risk of incident dementia. As shown in Table 2, higher proportion of participants with PRISm developed dementia compared to participants with normal spirometry, while COPD patients did not. Compared with participants with normal spirometry, participants with PRISm exhibited a higher risk of all-type dementia (Model 2 hazard ratio [HR], 2.70; 95% confidence interval [CI], 1.53–4.75), while subjects with COPD did not ($HR_2$, 1.03; 95% CI, 0.61–1.74), after accounting for all covariates. After being adjusted for propensity score, age and sex, results of model 3 were similar to model 2 (Table 2). Hazard ratios of association of PRISm and COPD with all-type dementia were 2.47 (95% CI, 1.40–4.35) and 1.08 (95% CI, 0.63–1.83), respectively.

Concurrently, participants with PRISm were also at increased risk of AD, albeit this did not reach statistical significance ($HR_2$, 1.87; 95% CI, 0.92–3.81). COPD was not significantly associated with AD ($HR_2$, 0.87; 95% CI, 0.48–1.59) (Table 2).

We also investigated the risk of developing dementia associated with lower lung function by using continuous parameters ($FEV_1$%, FVC%, $FEV_1$/FVC%) and their categorized quartiles (Figure 2). A lower value in $FEV_1$% predicted was associated with an elevated risk of all-type dementia ($HR_2$, 1.12; 95% CI, 1.02–1.23). Relative to participants with the highest FVC% predicted values (Quartile 4), those with the lowest FVC% predicted values (Quartile 1) were at increased risk of both all-type dementia (Model 2 hazard ratio [$HR_2$], 2.28; 95% confidence interval [CI], 1.31–3.98) and AD ($HR_2$, 2.13; 95% CI, 1.13–4.02), after accounting for demographics and *APOE* genotypes. A lower value in FVC% predicted was significantly associated with an increased risk of both all-type dementia and AD in all models. $FEV_1$/FVC was not associated with dementia risk in any model (Figure 2).

Moreover, a competing risk model was used to measure the competing risk of mortality during the follow-up period on the observation of dementia events. Although participants with PRISm suffered from higher cumulative incidence of all-cause mortality than participants with normal spirometry, participants with PRISm still exhibited significantly higher cumulative incidence of all-type dementia (P=0.018), but not of AD (P>0.05). (Supplementary Figure 1)

**Stratified analysis**

Methods and figures on the stratified analyses are presented in the online supplement. Regarding the association between COPD or PRISm and the risk of incident dementia, stratified analyses were performed in women, men, smokers, non-smoking participants, participants without history of stroke and diabetes, and APOE-e4 non-carriers. Significant associations were found between PRISm and all-type dementia in men (adjusted HR = 5.29, 95% CI, 2.40-11.65) – but not in women (adjusted HR = 1.65, 95% CI, 0.71-3.87) –, current or former smokers

(adjusted HR = 3.36, 95% CI, 1.71-6.60) – but not in never-smoking participants (adjusted HR = 1.95, 95% CI, 0.68-5.57) – , and in participants without a history of stroke (adjusted HR = 2.58, 95% CI, 1.45-4.59) and diabetes (adjusted HR = 2.56, 95% CI, 1.38-4.78) –, and participants without APOE-e4 allele (HR = 1.56, 95% CI, 0.71-3.45). Significant association between PRISm and AD risk were only observed among men. (Supplementary Figure 2)

We have tested the effect of interaction of lung function and sex, and interaction of lung function and smoking status in cox models, respectively, which tests for multiplicative interaction. These tests of interaction did not reach statistical significance. (data not shown)

In addition, supplementary Figure 3 shows the association between continuous spirometry parameters with the risk of newly diagnosed dementia (Supplementary Figure 3A) or newly diagnosed Alzheimer's disease (Supplementary Figure 3B), stratified by sex, smoking status and absence of stroke, diabetes and APOE-e4 non-carriers. A lower $FEV_1$% predicted was associated with a greater risk of all-type dementia only among women, never-smoking participants and those without prior stroke, but not among men, current or former smoking participants and participants without prior diabetes and APOE-e4 non-carriers. A lower FVC% predicted was associated with an increased risk of all-type dementia among all subgroups except APOE-e4 non-carriers. Statistical significance was not found between decreased $FEV_1$/FVC and risk of all-type dementia. Regarding elevated risk of AD, reduced FVC% predicted and $FEV_1$/FVC elevated were associated with AD among women, nonsmokers and those without prior stroke, while $FEV_1$% predicted did not show an increased risk of AD among those without prior stroke.

## Discussion

In this population-based cohort study, individuals with PRISm were at increased risk of all-type dementia, while those with COPD were not. Especially, predicted FVC% was strongly associated with a higher risk of dementia among the whole study population.

The main finding of this study is that PRISm was associated with an increased risk of dementia. Comorbidities, such as diabetes and stroke are more common among participants of this restrictive lung-function pattern [62], and may confound the link with impaired cognition and the increased risk for dementia. However, while we found a higher prevalence of prior stroke in participants with PRISm, the association between PRISm and dementia persisted after adjusting for these comorbidities. There are several possible mechanisms linking PRISm with dementia. Firstly, ambient pollution and inhalational exposures are associated with higher risk of PRISm [63], which could also contribute to the development of dementia [64, 65]. For example, fine particulate matter in air could not only lead to impaired lung function through disturbing alveolarization process and altering lung elastance at an earlier life stage [66], but also be linked to higher dementia risk via accumulation of Aβ42 and alteration on neuro-inflammation and brain immune response, as exposure to certain level of air pollution could upregulate expression of mRNA COX2 and IL-1β in olfactory bulb, disrupt tight junctions in frontal blood-brain barrier and activate nuclear NF□B in brain endothelial cells [65, 67].

Secondly, some studies reported that FVC decline in subjects with PRISm was accompanied with systemic inflammation [68-70]. Systemic inflammation in turn may be linked with cognitive impairment and/or occurrence of dementia [71]. Serum inflammatory cytokines, like (IL)-18, IL-1 receptor antagonist and IL-6, have been linked with AD [72], and high levels of serum IL-6 were associated with a greater risk of non-AD dementia as well [73]. Unfortunately, we did not have inflammatory markers available in this population to test this hypothesis.

Thirdly, reduced lung function could limit peak oxygen uptake and oxygen saturation, resulting in potential hypoxia [40, 74, 75]. In turn, hypoxia has been reported to induce cognitive deficiency and dementia in both human and animal studies [76, 77]. Mice with hypoxia exhibited tau hyperphosphorylation, Aβ upregulation and dysfunction of neurotransmitter system [77].

In stratified analyses, we found that the association between PRISm and dementia was present in men, current and past smokers, and participants without history of stroke and diabetes.

Though speculative, sex differences can potentially be explained by unmeasured confounding by sex hormones [78, 79]. Indeed, estrogen has protective effects on systemic and cerebrovascular atherosclerosis, which in turn impact both lung function and dementia risk [58, 78]. In this population-based study, we could not corroborate this speculation and future research is therefore needed to explore these hypotheses further.

The effect modification by smoking status indicates that the effect of poor lung function on risk of dementia is further aggravated in presence of smoking. This may be related to direct toxic effects of smoking in the brain, for instance increased levels of oxidants and free radical species, which promotes formation of senile plaque and neurofibrillary tangles. In turn, these pathological processes may interact with cerebral hypoxia and hypoperfusion due to poor lung function [80, 81].

With respect to stroke, APOE-e4 carriership and diabetes, we only had sufficient power to show the largest stratum and found that associations among persons without stroke, APOE-e4 non-carriers and non-diabetics remained largely similar to the overall population.

Among continuous lung function parameters, FVC% predicted, but not FEV1/FVC ratio or $FEV_1$% predicted, was significantly associated with both all-type dementia and AD risk. Previous studies have varyingly reported on FEV1, FEV1/FVR ratio, or FVC% predicted to be associated with dementia. Heterogeneity across study population, including differences in age-

range, sampling strategy and co-morbid conditions may explain differences in the strength of associations of the various parameters with dementia.

We did not demonstrate an association between COPD and the risk of dementia, in contrast to the prior study [48]. Previously, we found participants with PRISm and COPD to suffer from increased all-cause and cardiovascular mortality [41], and similarly the present competing risk model suggested the highest figure of all-cause mortality in COPD group. Therefore, mortality may hinder the occurrence of incident dementia during the follow-up period.

**Strengths and limitations**

An important strength of this study is the relatively large number of elderly participants included for assessment of the lung function through standardized protocols and dementia data based on continuous follow-up. Competing risks is a limitation when using traditional cox proportion-hazard regression analyses. However, we used competing risk model to calculate cumulative risk of dementia to correct effect of variable of interest. The small number of incident dementia cases limited our study power, but we applied propensity scores to avoid potential overfitting problem with adjustment for extensive covariates.

**Conclusions**

As a conclusion, among this community-dwelling population, participants with PRISm or participants with a low FVC% predicted lung function were at increased risk of dementia, compared to those with normal spirometry or a higher FVC% predicted, respectively. Further research is needed to elucidate whether this association is causal and how PRISm might contribute to dementia pathogenesis. Therefore, it is necessary to recognize PRISm and evaluate status of FVC% predicted when conducting spirometry tests in clinical settings.

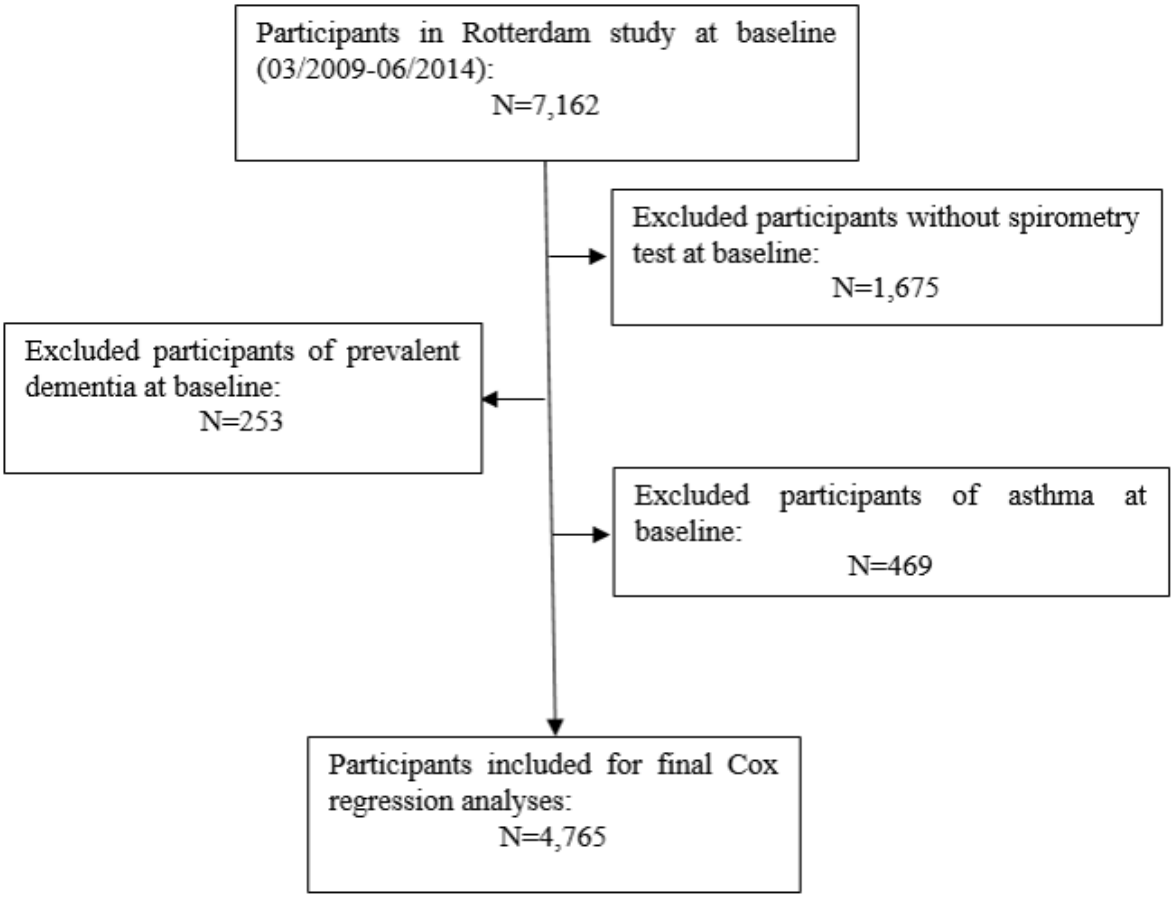



Figure 1. Flow chart for participants with interpretable spirometry at baseline, informed consent for follow-up and graph for definition of lung function categories.

Table 1. Baseline characteristics of participants, stratified by lung function category

| | Normal | PRISm | COPD | P value |
|---|---|---|---|---|
| n (%) | 3683 (77.3) | 319 (6.7) | 763 (16.0) | - |
| Age, years | 67.8 (12.5) | 68.6 (14.4) | 70.6 (13.4) | <0.001 |
| Female, (%) | 2120 (57.6) | 171 (53.6) | 324 (42.5) | <0.001 |
| Education level | | | | |
| Primary education | 246 (6.8) | 31 (9.9) | 81 (10.7) | <0.001 |
| lower education | 1445 (39.7) | 117 (37.1) | 279 (36.9) | |
| Intermediate education | 1089 (29.8) | 93 (29.5) | 249 (32.9) | |
| Higher education | 864 (23.7) | 74 (23.5) | 148 (19.6) | |
| Smoking status, (%) | | | | |
| Never | 1383 (37.6) | 97 (30.4) | 135 (17.7) | <0.001 |
| Former | 1960 (53.2) | 177 (55.5) | 415 (54.4) | |
| Current | 340 (9.2) | 45 (14.1) | 213 (27.9) | |
| Systolic pressure | 141 (29) | 142 (29) | 142 (26) | 0.369 |
| Body mass index, kg/m2 | 27.0 (5.0) | 28.4 (5.9) | 26.1 (5.0) | <0.001 |
| Triglycerides, mg/dl | 1.3 (0.7) | 1.4 (0.9) | 1.2 (0.7) | <0.001 |
| History of stroke | 34 (0.9) | 7 (2.2) | 12 (1.6) | 0.040* |
| History of diabetes mellitus | 292 (8.0) | 31 (10.0) | 73 (9.7) | 0.200 |
| Apolipoprotein E genotype, (%) | | | | |
| ε4-allele positive | 937 (27.3) | 66 (22.8) | 196 (27.8) | 0.142 |
| ε4-allele negative | 2496 (72.7) | 224 (77.2) | 509 (72.2) | |
| FEV1/FVC | 78.7 (6.4) | 76.1 (7.1) | 65.6 (7.6) | <0.001 |
| FEV1% predicted | 103.2 (18.7) | 73.8 (10.6) | 79.1 (24.7) | <0.001 |
| FVC% predicted | 101.2 (17.9) | 72.2 (11.7) | 94.0 (24.9) | <0.001 |

Definition of abbreviations: COPD = Chronic Obstructive Pulmonary Disease; FEV1 = Forced Expiratory volume in one second; FVC = Forced Vital Capacity; PRISm = Preserved Ratio Impaired Spirometry. Data represent original data without imputed values. Missing values were present for education attainment (1.0%), systolic blood pressure (2.8%), triglyceride (1.7%) and history of diabetes (7.1%).

*Fisher's exact test

Table 2. Lung function category and risk of dementia

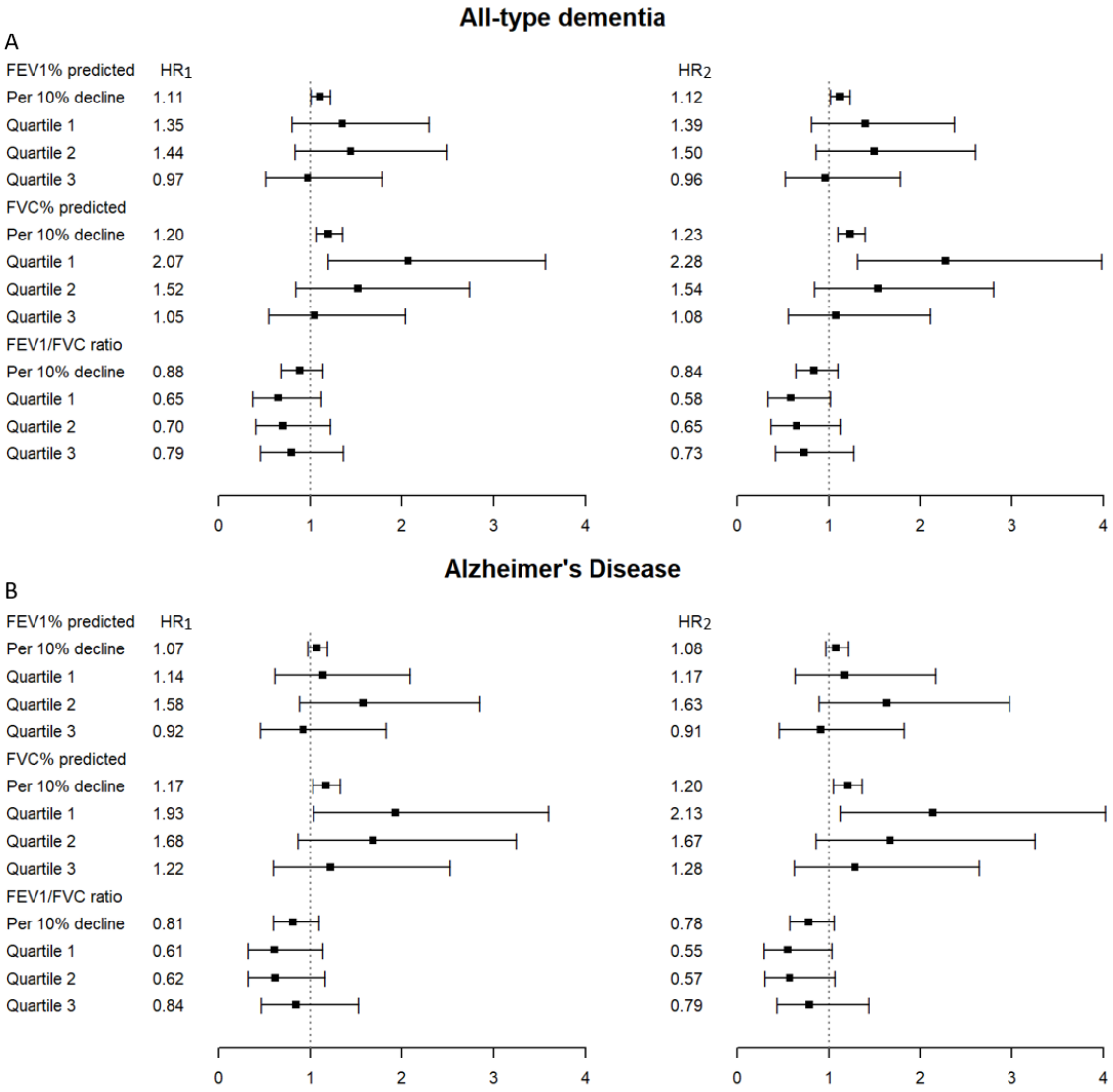
| | All-type dementia | | | | |
|---|---|---|---|---|---|
| | cases/death/N | FU, years | HR1 (95% CI) | HR2 (95% CI) | HR3 (95% CI) |
| Normal | 75/179/3683 | 3.3 (1.6) | 1.0 | 1.0 | 1.0 |
| PRISm | 15/25/319 | 3.4 (1.6) | 2.42 (1.38;4.24) | 2.70 (1.53;4.75) | 2.47 (1.40;4.35) |
| COPD | 20/88/763 | 3.4 (1.6) | 1.06 (0.63;1.77) | 1.03 (0.61;1.74) | 1.08 (0.63;1.83) |

| | AD | | | | |
|---|---|---|---|---|---|
| | cases/death/N | FU, years | HR1 (95% CI) | HR2 (95% CI) | HR3 (95% CI) |
| Normal | 65/179/3673 | 3.3 (1.6) | 1.0 | 1.0 | 1.0 |
| PRISm | 9/25/313 | 3.4 (1.6) | 1.70 (0.84;3.43) | 1.87 (0.92;3.81) | 1.74 (0.86;3.54) |
| COPD | 15/88/758 | 3.4 (1.6) | 0.89 (0.49;1.60) | 0.87 (0.48;1.59) | 0.89 (0.49;1.63) |

Definition of abbreviations: AD=Alzheimer Disease; COPD = Chronic Obstructive Pulmonary Disease; CI=Confidence Interval; FU = Follow-up; HR = Hazard Ratio; PRISm = Preserved Ratio Impaired Spirometry; Model1 = Cox regression adjusted for *APOE* genotype, age, sex and education level; Model2 = Model1 plus adjustment smoking status, BMI, systolic blood pressure, triglycerides and history of comorbidities (stroke and diabetes mellitus); Model3 = Cox regression adjusted for propensity scores*, age and sex.

* Propensity scores was calculated with age, sex, education level, smoking status, BMI, systolic blood pressure, triglyceride, history of comorbidities (stroke and diabetes mellitus) and *APOE* genotype; follow-up time started after spirometry at baseline.

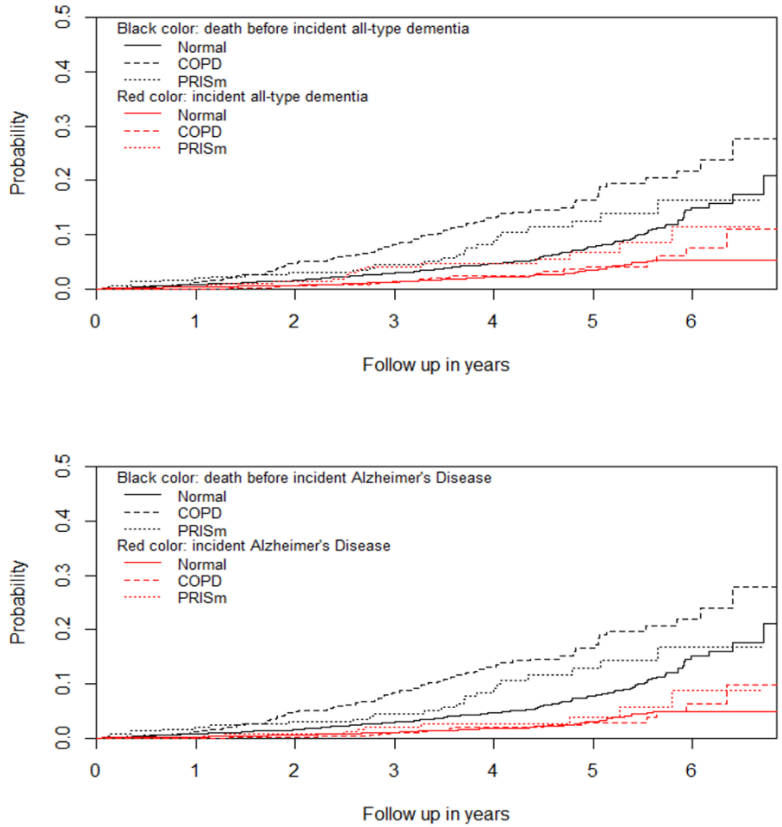
Figure 2. Respiratory indexes ($FEV_1$% predicted, FVC % predicted and $FEV_1$/FVC ratio) and risk of dementia (A) and Alzheimer's disease (B)




Definition of abbreviations: AD=Alzheimer Disease; A = All-type dementia; B = Alzheimer's disease; CI=Confidence Interval; HR = Hazard Ratio; $FEV_1$ = Forced Expiratory volume in one second; FVC = Forced Vital Capacity; HR1 = HR from Cox Proportional-Hazard regression analysis adjusted for *APOE* genotype, age, sex and education level; HR2 = HR1 with additional adjustment for current or ever smoking, BMI, systolic blood pressure, triglyceride and history of comorbidities (stroke and diabetes mellitus). Participants in the highest percentile (Quartile 4) of spirometry indexes were regarded as reference group (hidden).

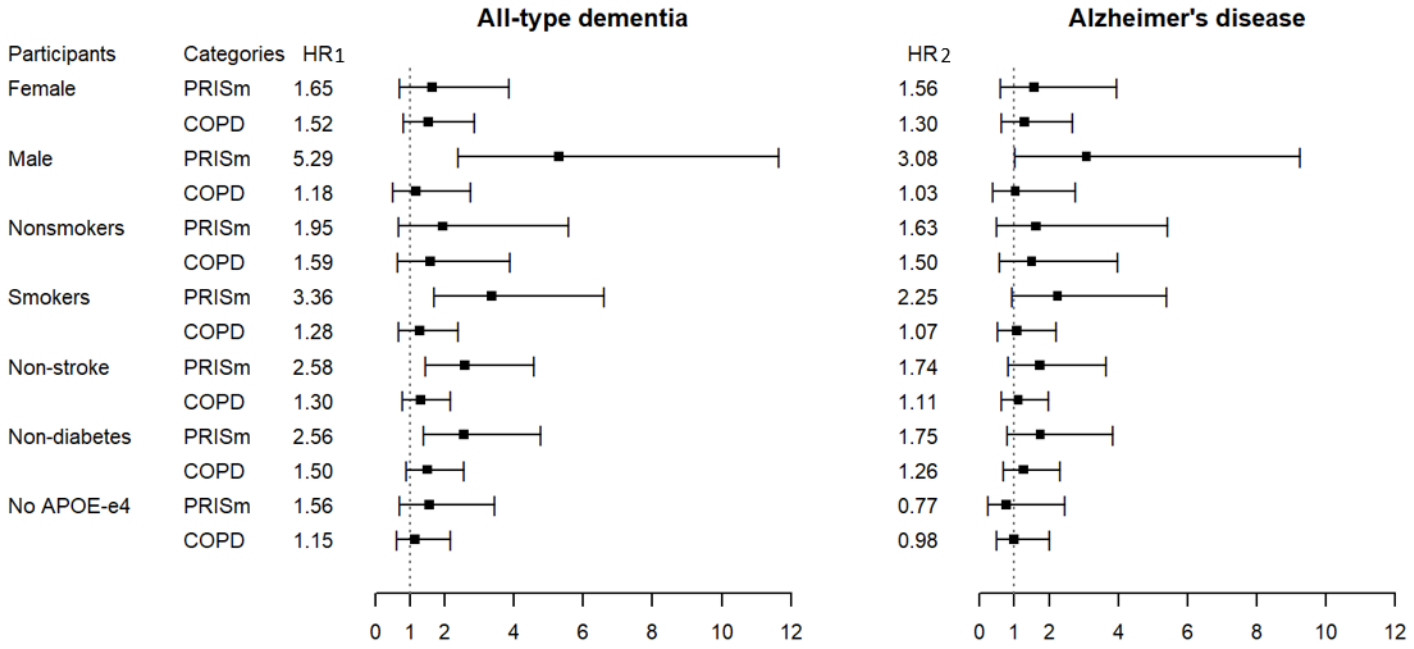
* follow-up time start after spirometry at baseline

Supplementary Figure 1. Cumulative incidence curves for all-cause mortality and incident dementia according to lung function rank



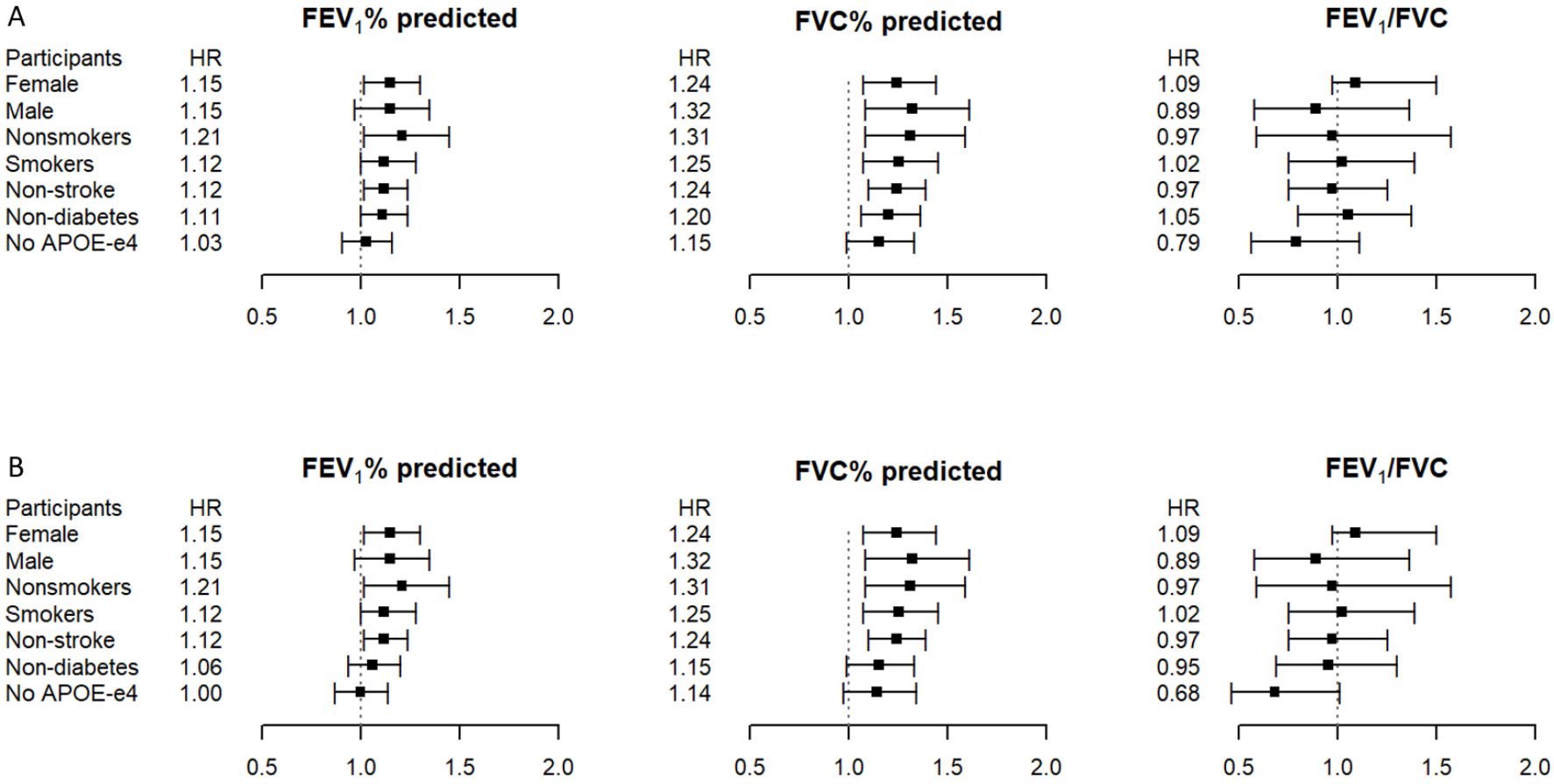

Definition of abbreviations: PRISm = Preserved Ratio Impaired Spirometry; COPD = Chronic Obstructive Pulmonary Disease; AD = Alzheimer's disease; * follow-up time start after spirometry at phase.

Supplementary Figure 2. Lung function impairment and risk of incident dementia among specific types of population




Definition of abbreviations: HR = Hazard Ratio; PRISm = Preserved Ratio Impaired Spirometry; COPD = Chronic Obstructive Pulmonary Disease; Model = Cox regression adjusted for APOE genotype (except for stratified analyses among non-APOE-e4 carriers); Nonsmokers = participants without history of smoking; Smokers = former or current smokers; Non-stroke = participants without stroke; Participants with normal spirometry (hidden) were regarded as reference group.* follow-up time start after spirometry at phase.

Supplementary Figure 3. Per 10% decline in different respiratory indexes and risk of incident dementia among specific types of population



Definition of abbreviations: HR = Hazard Ratio; A = All-type dementia; B = Alzheimer's disease; $FEV_1$ = Forced Expiratory volume in one second; FVC = Forced Vital Capacity; Model = Cox regression adjusted for APOE category; Nonsmokers = participants without history of smoking; Smokers = former or current smokers; Non-stroke = participants without stroke; * follow-up time start after spirometry at phase.

# Chapter 2.2 Lung Function Impairment In Relation To Cognition and Vascular Brain Lesions: The Rotterdam Study

**Abstract**

**Objective**

To investigate the association of chronic obstructive pulmonary disease (COPD) and Preserved Ratio Impaired Spirometry (PRISm) with cognitive performance and presence of vascular brain lesions (VBL).

**Methods:** We determined both cross-sectional and longitudinal association of lung function impairment with cognition, as well as cross-sectional association of lung function impairment with VBL, in the general population. Between 2009 and 2014 we included 3,941 participants from the Rotterdam Study with spirometry tests, brain MRI scans and cognition tests, of whom 1,815 had follow-up data on cognition.

**Results:** Our finding indicated that cross-sectionally, participants with PRISm or COPD GOLD2-4 had worse global cognitive performance. We did not find differences in cognition over time between those with normal spirometry versus those with lung function impairment. In addition, PRISm and COPD GOLD2-4 was associated with higher prevalence of lacunar infarcts compared to normal spirometry.

**Conclusions:** This study suggests that persons with COPD GOLD2-4 or restrictive lung function, defined as PRISm, are characterized by poorer global cognitive function and higher prevalence of lacunar infarcts.

**Keywords:** PRISm; lung function; COPD; vascular brain lesions; cognition

## Introduction

Dementia is a major public health issue [36, 82], but before the clinical onset of this condition, patients in the preclinical stage experience a period of accelerated cognitive decline, which is further preceded by pathological changes in the brain [83]. Elucidating etiological pathways and mechanisms preceding cognitive decline is crucial in order to identify persons who are at risk for dementia, and who may benefit from preventive interventions. Previous studies shown the strong impact of vascular risk factors and systemic vascular disease on cognitive decline and corresponding changes on brain MRI, i.e. vascular brain lesions (VBL) [84-87]. In this regard, several studies have explored the link between lung function impairment and cognitive decline [88]. Possible explanations for this association include chronic hypoxia and shared risk factors, such as smoking.

While most studies have focused on COPD, lung function impairment in the elderly may also reflect a restrictive lung pattern, often termed PRISm. Only recently, studies have started investigating PRISm more extensively with reported prevalences ranging from 3% to 20% in the elderly [89]. Possible systemic effects of PRISm are thought to include similar pathways as for COPD. Yet, the impact of PRISm on cognitive decline remains largely unexplored, although in previous study we tested the effect of lung function impairment on dementia [90]. One study using spirometry showed that lung function impairment suggestive for a restrictive ventilatory pattern is associated with impaired cognitive function, but their definition for restrictive spirometry differed from the formal definition of PRISm [51, 89].

In this study, we aimed to investigate the association of COPD and PRISm with cognitive performance cross-sectionally and longitudinally. We also explored cross-sectional association between lung function impairment and presence of VBL.

## Methods

This study was conducted within the Rotterdam Study, a prospective cohort study that started in 1990, comprising almost 15,000 participants aged at least 45 years, with the aim of studying chronic diseases in the general population [91]. Every four to five years, participants underwent follow-up examinations, consisting of a home interview and various physical examinations at the research center. MRI scanning was introduced in to the core protocol of the study from 2005 onwards and spirometry from 2009 onwards. Participants with dementia or asthma at baseline were excluded. A total of 3,941 participants had undergone interpretable spirometry, MRI scan and cognition tests that visited the research center at baseline between 2009 and 2014. After excluding 151 participants with poor testing status, 3,790 participants with at least one individual cognitive tests were included for cross-sectional analyses of lung function and cognition. Of these 1,815 participants attended the follow-up examinations with good testing status (from 05/2014 to 05/2016) and thus were eligible for observation of cognitive changes over time. Additionally, 3,941 participants at baseline were included for cross-sectional analyses of lung function and presence of microbleeds, lacunar infarcts and cortical infarcts, of whom 3,801 participants were included for exploring association of lung function with volume of white matter lesions cross-sectionally after excluding 140 participants with cortical infarcts. When measuring volumes of white matter, grey matter, or brain lesions, it is important to exclude scans with cortical infarcts, because the tissue segmentation is unreliable in these cases. Thus, we had to remove scans with cortical infarcts to get robust and exact measurements of sizes of white matter lesions, and further explore associations of lung function impairment with white matter lesions. (Figure 1).

### *Spirometry testing*

Lung function was assessed via pre-bronchodilator spirometry performed by trained paramedical personnel using a Master Screen PFT Pro (Care Fusion, Netherlands) according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines [46]. Predicted forced vital capacity (FVC) and predicted forced expiratory volume in one second ($FEV_1$) values were calculated using Global Lung Initiative (GLI) reference equations taking age, sex, height and ethnicity into account [47]. COPD1 (GOLD stage 1 defined as $FEV_1/FVC<70\%$ and $FEV_1 \geq 80\%$ predicted), COPD2-4 (GOLD stage 2-4 defined as $FEV_1/FVC<70\%$ and $FEV_1<80\%$ predicted), PRISm (defined as $FEV_1/FVC \geq 70\%$ and FEV1<80% predicted) and normal spirometry (defined as $FEV_1/FVC \geq 70\%$ and an $FEV_1 \geq 80\%$ predicted) were distinguished [89, 92].

***Cognitive testing***

Participants underwent the same cognitive tests at the baseline and at follow-up examinations: Stroop test, Letter-Digit Substitution Task (LDST), Word Fluency Task (WFT), 15-Word Learning Test of delayed recall, immediate recall and recognition (WLTdel, WLTimm, WLTrecog) and Purdue Pegboard test (PPB test) [93]. The Stroop Test measures attention and concentration and consisted of three trials. In trial 1, the card contains color names printed in black and participants are asked to name the printed word. In trial 2, the card contains colored blocks and participants are asked to name the printed color. In trial 3, the card contains color names printed in a different color than the color name and participants are asked to name the color of the ink. The outcome variable is the time needed to finish the trial. The LDST was used to measure processing speed. Participants make as many letter-digit combinations as possible within 60 seconds, following an example that shows the correct combinations. The WFT was used to test verbal fluency. Participants are asked to name as many animals as possible within 60 seconds. The WLT tests memory functions with immediate recall and delayed recall

components. Participants are given a list of 15 unrelated words repeated over five different trials and are asked to repeat them. To test 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. Free delayed recall was tested 15 minutes later. 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 if they recognized the words as the ones presented to them during the immediate recall trial [93, 94]. The PPB is a measure of unilateral and bilateral fine manual dexterity and consists of a pegboard with two parallel rows of 25 holes and a number of pins. The participants were asked to place as many pins as possible into the holes on the board in a prescribed order, within 30 seconds. The test consists of 3 trials: placing the pins using the right hand only; using the left hand only; using both hands. Outcome variable was the number of pins placed correctly in every trial [95].

The distribution of all tests was transformed into a normal standardized distribution and a Z-score for every individual was calculated at baseline and follow-up. Z-score were calculated by individual raw scores minus the mean value of whole population, divided by the population standard deviation. We constructed a compound score (G-factor) for global cognitive function as the average of all individual tests. The G-factor is the first component of the principal component analysis. For tests with multiple subtasks, only one subtask was included in order to prevent highly correlated tasks distorting the factor loadings [94]. These tests contained Stroop test (color naming test), LDST, WFT, WLTdel and PPB test. Of these 3,790 persons with at least one individual cognitive test, 3,134 participants underwent all subgroup cognitive tests needed for assessing G-factor at baseline. Correspondingly, among 1,815 participants with follow-up data on cognition, 878 participants obtained G-factor.

***MRI protocol and image processing***

MRI of the brain was performed on a 1.5T scanner (General Electric Healthcare, Milwaukee, WI) using an 8-channel head coil. Imaging acquisition included a high-resolution axial T1-weighted sequence, a fluid-attenuated inversion recovery sequence, a proton density–weighted sequence, and a T2*- weighted gradient echo sequence. Total brain volume was quantified by automatic tissue segmentation. Details about the sequences, preprocessing, and the classification algorithm have been described previously [96]. All segmentation results were visually inspected and manually corrected if needed. All scans were appraised by trained researchers for the presence of cerebral microbleeds (i.e., small round to ovoid hypointense areas on T2*-weighted images), lacunar infarcts (i.e., focal lesions ≥3 and <15 mm) and cortical infarcts, as well as volumes of white matter lesions. These ratings were done blinded to clinical data [97].

***Covariates***

Demographic information included age, sex, education level (primary education, lower education, intermediate education, higher education), smoking status (never, former, current), systolic blood pressure (mmHg), body mass index (BMI, kg/m$^2$, calculated by weight [kg] divided by height [m] squared), serum lipids (triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C)) [mmol/L], history of chronic comorbid conditions (diabetes, stroke and depressive symptom, chronic kidney disease, atrial fibrillation, heart failure, and coronary heart disease), and concomitant medications (antihypertensives, diuretics, vasoprotectives, betablocking agents, calcium blockers, ACE-inhibitors, serum lipid reducing agents and statins), which are associated with cognitive impairment or dementia [30, 37, 98-100]. Diabetes was defined as a fasting plasma glucose level ≥ 7 mmol/L, a non-fasting plasma glucose level ≥ 11.1 mmol/L or the use of blood glucose lowering medication [101]. Stroke was defined according to the World Health Organization criteria [102]. Depressive symptoms were

assessed with validated version of the Centre for Epidemiologic Studies Depression (CES-D) scale (range:0-60) [103]. Scores of 16 or greater are regarded as suggestive of clinically significant depressive symptoms [103]. Coronary artery disease (CHD) were defined as the presence of myocardial infarctions, all CHD mortality or revascularization [104]. Heart failure (HF) was defined in accordance with the European Society of Cardiology [105]. Atrial fibrillation (AF) was verified by two physicians using all 12-lead ECGs along with the Modular ECG Analysis System (MEANS) [106-108]. Blood samples were extracted for determination of levels of triglycerides and DNA at the research center. Apolipoprotein E (*APOE*) genotype was determined using a PCR in the original cohort (RS-I, starting between July, 1989 and September, 1993) and a bi-allelic TaqMan assay (rs7412 and rs429358) on labeled DNA samples in the two cohorts (RS-II-3, starting between February, 2000 and December, 2001; and RS-III-2, starting between February, 2006 and December, 2008), respectively. This study included these three sub-cohorts. *APOE*-ε4 represented carrier of one or two ε4 alleles. With regard to dose-dependent effect of *APOE*-ε4 allele on cognition [109, 110], participants were categorized into three groups: without *APOE*-ε4 allele (ε2ε2, ε3ε3 or ε2ε3 genotypes), with *APOE*-ε4 heterozygotes (ε2ε4 or ε3ε4) and with *APOE*-ε4 homozygotes (ε4ε4). As the strongest genetic risk factor for dementia [109], *APOE* genotype has additionally potent cardiovascular effects, including arteriosclerosis and cardiac function. In this regard, *APOE* genotype may also impact lung function. We therefore included *APOE* genotype in the models as possible confounder [48, 51]. Imputation was not conducted on missing covariates due to missing at completely random and very low missingness proportion (<5%). (Table 1)

**Statistical analysis**

Data are expressed as median (interquartile range [IQR]) for continuous variables among subgroups of different lung function categories. Categorical variables were compared with

Pearson Chi square test or Fisher exact test. Continuous variables were tested by using Kruskal-Wallis test for more than two group comparisons.

We determined the cross-sectional association of lung function impairment with cognitive function at baseline using linear regression analyses. We calculated average differences in G-factor and z-scores of individual cognitive tests between subgroups with different lung function (groups with normal lung function as reference) through Tukey all-pair comparisons method based on ANOVA models. 3) In order to assess associations of G-factor of cognition with lung function parameters, these parameters were further categorized into quintiles with the lowest quartile as reference. Cross-sectional analyses were adjusted for age, sex, education, smoking status, BMI, systolic blood pressure, serum lipids (triglycerides, total cholesterol, HDL cholesterol) , history of comorbidities (stroke, diabetes, depressive symptom, atrial fibrillation, heart failure, coronary heart disease), medications (antihypertensives, diuretics, vasoprotectives, beta-blocking agents, calcium blockers, ACE-inhibitors, serum lipid reducing agents and statins), and *APOE* genotype. Longitudinal association of lung function with cognition were also determined using linear regression models with adjustment of same covariates above in cross-sectional analyses and additionally for baseline test scores and the time between two cognitive tests. The time between two cognitive tests were calculated with the formula: $Time_{gap} = (Dates_{follow\text{-}up} - Dates_{baseline}) / 365.24$.

Then, we determined the cross-sectional association between lung function impairment and the presence of microbleeds, lacunar infarcts and cortical infarcts, using logistic regression models. Similarly, average differences in volumes of white matter lesions (WML) between different subgroups were obtained using above methods in cognition analyses. In order to meet requirement of normal distribution, we used log transformation for original values of WML volume. Cross-sectional analyses were adjusted for the covariates above in cross-sectional analyses of cognition.

Covariates to be included as possible confounders were defined based on clinical relevance and literature knowledge [30, 37, 98-100, 111-115].

In addition, we conducted stratified analyses in women, men, non-smoking participants, and participants without history of stroke, diabetes and depressive symptoms, as these were most common conditions in general population and were reported to modify the association on the basis of current literature and biological plausibility [58, 99, 116-118]. For example, estrogen, as a major sex hormone, plays an important role in preventing systemic and cerebrovascular atherosclerosis, which conversely influence both lung function and cognition [58, 78, 79, 119]. Stratified analyses may provide detailed information on the association between lung function and cognition among different population.

In sensitivity analysis, we additionally performed binomial logistic regression for analyses of effect of lung function on cognitive change. For some cognitive tests, including LDST, WFT, WLTdel, WLTimm, WLTrecog and PPB test, cognitive decline was defined as a drop in following-up scores of more than mean difference between baseline and following-up tests per year [$Scores_{drop/year} > |(Scores_{follow\text{-}up} - Scores_{baseline}) / (N_{participants}*Time_{gap})|$]. On the contrary, cognitive decline was defined as an increase in following-up scores of more than mean difference in Stroop tests [$Scores_{increase/year} > |(Scores_{follow\text{-}up} - Scores_{baseline}) / (N_{participants}*Time_{gap})|$]. Analyses were adjusted for similar covariates above in cross-sectional analyses for cognition.

A p value of $<0.05$ was considered statistically significant. Data analyses was done using R version 3.6.0 (Foundation for Statistical Computing, Vienna, Austria).

## Results

### *Characteristics of the study population*

Among 3,941 included participants (mean age 67.5±12.5 years, 53.7% women), 3080 (78.2%) had normal spirometry, 249 (6.3%) had PRISm, 304 (7.7%) had COPD GOLD stage 1 and 308 (7.8%) had COPD GOLD stage 2-4. (Table 1)

Participants without follow-up were younger (62.3 versus 74.2 years, P<0.001), had less impaired spirometry (20.4% vs 22.6%, P=0.002) and showed higher scores in individual cognitive tests, compared to participants with follow-up. (S.Table 1)

### *Lung function and cognition*

Table 2 presents the association between lung function impairment and cognitive performance. Cross-sectional analyses revealed that lung function impairment was associated with poor performance on several cognitive tests in the cross-sectional analyses (n=3,790). (Table 2A)

In the cross-sectional analyses, we found that PRISm was associated with lower global cognition scores [mean difference in G-factor score compared to normal spirometry: –0.27 (95% confidence interval (CI): –0.43;–0.11)] and scores of LDST, WFT and PPB test. Similarly, COPD GOLD 2-4, but not COPD GOLD 1, was associated with poorer performance on global cognition, LDST, both WLT immediate and delayed recall and PPB test, compared with participants with normal spirometry.

Longitudinal analyses performed in 1,815 individuals did not reveal any statistically significant associations. (Table 2B)

We also tested the association between lung function parameters and cognitive performance (Figure 2). Higher values in $FEV_1$% predicted and FVC% predicted were associated with better global cognitive function in the cross-sectional analyses (Figure 2A). Similar associations were not observed in longitudinal analyses (Figure 2B).

### *Lung function and VBL*

Cerebral microbleeds were present in 851 (21.6%) participants, lacunar infarcts in 355 (9.0%), and cortical infarcts in 140 (3.6%). (Table 1)

In the cross-sectional analyses, lacunar infarcts were significantly associated with PRISm (odds ratio [OR]: 1.65; 95% confident interval[95% CI]: 1.03;2.56), COPD GOLD 2-4 (OR: 1.78; 95% CI: 1.20;2.61) and lower values in $FEV_1$% predicted (OR: 1.17; 95% CI: 1.10;1.25), FVC% predicted (OR: 1.19; 95% CI: 1.10;1.29) and $FEV_1$/FVC (OR: 1.18; 95% CI: 1.01;1.38). Lung function impairment were not associated with cerebral microbleeds or cortical infarcts. As for WML, we found that lower values in FVC% predicted [MD: 0.02; 95% CI: 0.01;0.03)], but not in $FEV_1$% predicted or $FEV_1$/FVC, were associated with larger log-transformed volume of WML. We did not find significant associations of PRISm and COPD GOLD 2-4 with volume of WML. (Figure 3)

***Stratification analyses***

We stratified analyses for the association between lung function and cognition by sex, by smoking status and by history of stroke, diabetes and depressive symptoms.

Among women and subgroups without comorbidities, participants with PRISm or COPD GOLD 2-4 had lower scores in global cognitive function compared to participants with normal spirometry at baseline. And only PRISm is cross-sectionally associated with lower global cognition scores among both men and never-smoking subgroup. We found no significant association of lung function impairment with worse cognitive function in the stratified longitudinal analysis. (S.Figure 1)

***Sensitivity analyses***

S.Figure 2 shows effect of baseline lung function on individual cognitive decline, using binomial logistic regression. There were no statistically significant association between worse lung function impairment and more steep cognitive decline, compared to participants with normal spirometry. (S.Table 2)

## Discussion

In this study, individuals with lung function impairment, both PRISm and COPD GOLD 2-4 were characterized with poorer cognitive function, while those with COPD GOLD 1 were not. In addition, both PRISm and COPD GOLD 2-4 were also associated with presence of lacunar infarcts, on brain MRI.

In line with previous studies we found that moderate-to-severe COPD was associated with cognitive impairment [51]. Similarly, lower baseline lung function parameters, including $FEV_1$% predicted and FVC% predicted, were significantly associated with cognitive impairment. The link between COPD and cognitive impairment can be partly explained by shared underlying risk factor such as smoking, comorbidities and *APOE*-ε4. Although in our study we did not find differences in some comorbidities (stroke, diabetes and depression symptoms) and *APOE*-ε4 distribution across groups, we cannot fully rule out that there may still be residual confounding by *APOE* (as well as the other variables), explaining why adjustments for these factor(s) did not completely remove the effects found. However, after controlling for many potential confounders in our linear regression models, we still observed significant associations between PRISm or COPD GOLD2-4 and cognitive impairment. This suggests that we cannot exclude a possible direct effect of COPD on cognition, or the other way around, assuming there is no residual confounding. In case of a direct effect, chronic hypoxia as a consequence of lung function impairment may result in neurodegeneration [120, 121]. Severity of hypoxemia may get worse by increased ventilation/perfusion mismatch resulting from progressive airflow limitation in more severe COPD [122]. Alternatively, COPD may cause cognitive decline through inflammatory infiltrate. Some reported that increased percentage of lymphocytes and macrophages was weakly negatively correlated with decreased

scores of cognitive tests (MMSE) [123]. Conversely, poor cognition may influence cooperation during spirometry resulting in lower lung function values.

We also found that PRISm was associated with cognitive impairment. Herein, we are the first to study the association between PRISm and cognition. A previous US population-based study found restrictive lung function, characterized by decreased FVC and normal $FEV_1$/FVC ratio, was associated with cognitive impairment [51]. Possible mechanisms linking lung function impairment with dementia may include several different aspects. First, PRISm may result from harmful exposures, such as ambient pollution and inhalational exposures [63], at earlier life stage which has also been shown to lead to impairment of cognitive function [124]. Previous studies reported that lower FVC in subjects with PRISm was accompanied with systemic inflammation [68, 70, 125]. Inflammation has been observed to link to the pathogenesis of cognitive impairment [125]. For instance, several markers of inflammation, such as plasma fibrinogen, d-dimer and C-reactive protein, are associated with cognitive decline [126, 127]. Also, restrictive ventilator pattern has been reported to be associated with a higher incidence of diabetes, which is also a common risk factor of cognitive impairment and dementia [128]. However, we adjusted for diabetes in models and still found significant associations, implicating the association between PRISm and cognition to be independent of a shared etiology or at least beyond the factors controlled for.

In this study, we did not find a significant association between lung function impairment and accelerated cognitive decline when compared to those with normal spirometry. The lack of an association between lung function impairment at baseline and accelerated cognitive decline may be partly explained by selection bias. In this study, participants lost to follow-up were younger and had higher cognitive scores in most individual tests, and they may suffer from more fast accelerated cognitive decline over time, compared to those within follow-up. This indicates insignificant

declining magnitude between participants with lung function impairment and those with normal spirometry in longitudinal analyses. Although lung function impairment may not accelerate cognitive decline, participants with baseline lung function impairments still were exposed to high risk of incident dementia as found in previous work [90].

Another finding of this study is that impaired lung function (COPD GOLD 2-4 and PRISm) was associated with presence of lacunar infarcts, consistent to results of previous studies [129, 130]. A significant association of COPD GOLD 1 with VBL was not observed. Furthermore, the significant association of lower FVC with a higher number of VBL and larger volume of white matter lesions was found in this study and other studies [129, 131, 132]. Impaired lung function may be linked to development of VBL through reducing oxygen supply to brain, which could may aggravate harmful effect of ischemia on VBL pathogenesis [129, 133-135].

**Strengths and Limitations**

The most important strength of this study is the relatively large number of elderly participants recruited from the general population, that were included for the assessment of both lung function and neurocognitive ability through standardized protocols. Interpretable spirometry was guaranteed by strict adherence to the instruction of performing lung function test. Another strength also includes high-quality data of VBL detection derived from effective brain-imaging techniques, and the longitudinal follow-up with prospective information collection.

A limitation of this study is that COPD is a chronic disease, which is commonly accompanied with multiple comorbidities. Therefore, it is challenging to ascertain the effect of COPD on cognitive performance and development of VBL independent of other comorbidities. However, the associations of COPD and cognition, as well as presence of VBL, were independent of commonly known conditions, including diabetes, stroke and depressive symptoms. Another limitation is

cross-sectional design for exploring association between lung function impairment and presence of VBL. However, the significant associations of lower values in lung function parameters and presence of cerebral microbleeds and/or lacunar infarcts may indicate potential links between lung function impairment and VBL development. In addition, we do not have multiple repeated measurements of respiratory tests available in our study, except respiratory tests at baseline. In fact, longitudinal lung function assessment could better capture dynamic impact of lung function on cognitive change over time in real-world practice. Moreover, the third limitation is the small sample size in the longitudinal analysis. There are several reasons accounting for the limited number of participants in the longitudinal analysis as follows: 1) increased mortality with growing age from about 70 years at the baseline; 2) inability to perform the follow-up cognitive test due to frailty within follow-up; 3) higher prevalence of dementia with older age prevents cognitive test later on. The last limitation lies on strict spirometry test that requires good cognitive performance which may be a limitation because it may underestimate observing whether COPD causes cognitive decline.

**Conclusions**

As a conclusion, among this community-dwelling population, there was a cross-sectional association between lung function impairment and poor cognitive function, as well as presence of lacunar infarcts. Despite often being an ignored subgroup in pulmonary research and clinical practice, PRISm may play a role in the etiology of cognitive impairment and VBL development. More research is needed to elucidate whether this association is causal and how PRISm might contribute to pathogenesis of cognition impairment.

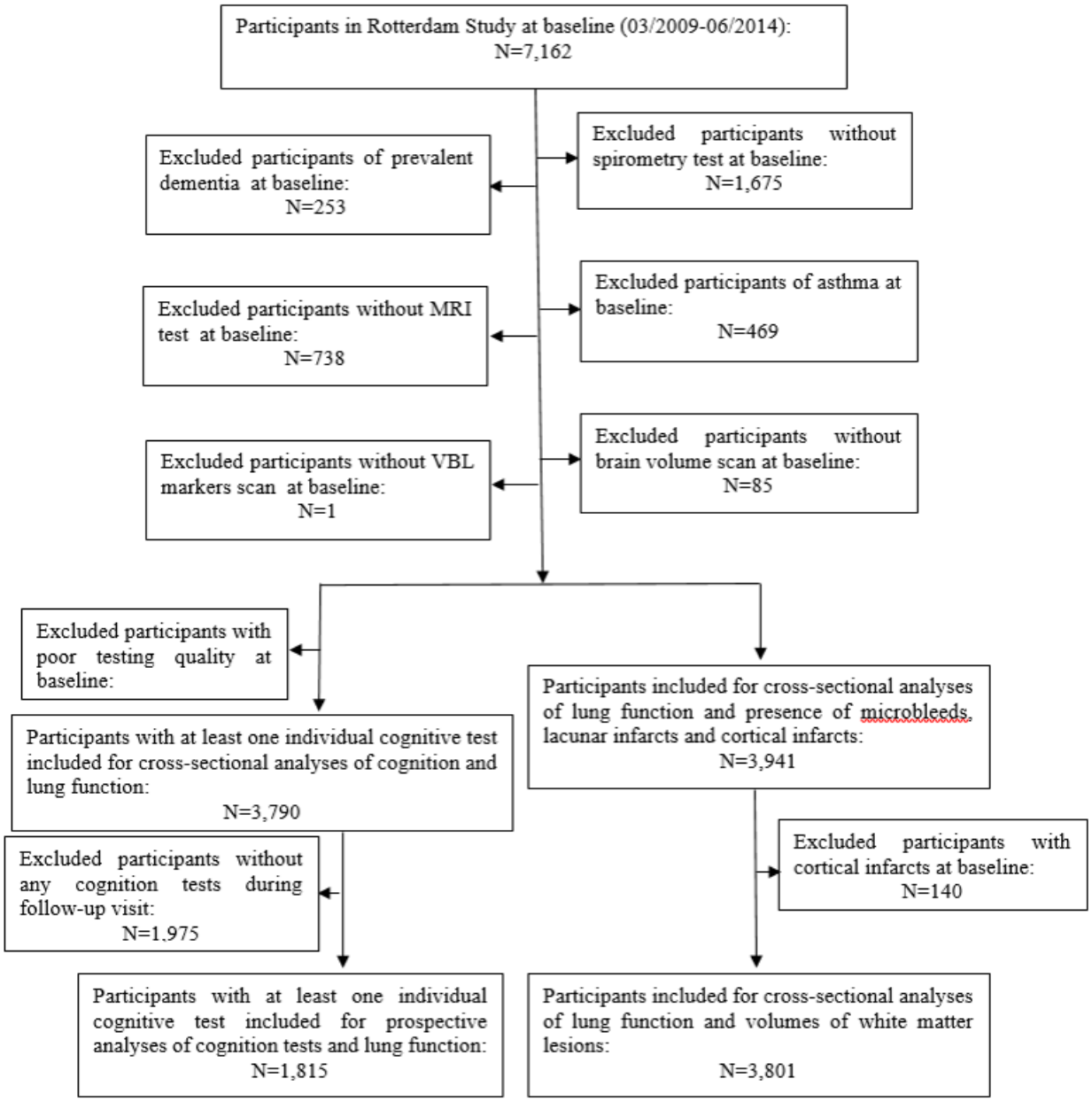



Figure 1. Flow chart for participants with interpretable spirometry at baseline and informed consent for follow-up. COPD = Chronic Obstructive Pulmonary Disease; PRISm = Preserved Ratio Impaired Spirometry; VBL = Vascular Brain Lesions.

Table 1. Baseline characteristics among participants with different lung function (N=3941)

| | Normal | PRISm | COPD1 | COPD2-4 | P Value |
|---|---|---|---|---|---|
| n (%) | 3080 (78.2) | 249 (6.3) | 304 (7.7) | 308 (7.8) | |
| Age, yr | 67.3 (12.3) | 67.9 (15.3) | 67.6 (12.1) | 71.0 (14.3) | **<0.001** |
| Female, % | 1730 (56.2) | 136 (54.6) | 122 (40.1) | 130 (42.2) | **<0.001** |
| Education level | | | | | |
| Primary education | 202 (6.6) | 25 (10.2) | 25 (8.3) | 38 (12.5) | **<0.001** |
| lower education | 1166 (38.3) | 90 (36.6) | 98 (32.3) | 121 (39.7) | |
| Intermediate education | 934 (30.7) | 70 (28.5) | 101 (33.3) | 101 (33.1) | |
| Higher education | 745 (24.5) | 61 (24.8) | 79 (26.1) | 45 (14.8) | |
| Smoking status, % | | | | | |
| Never | 1155 (37.5) | 83 (33.3) | 69 (22.7) | 35 (11.4) | **<0.001** |
| Former | 1633 (53.0) | 127 (51.0) | 164 (53.9) | 163 (52.9) | |
| Current | 292 (9.5) | 39 (15.7) | 71 (23.4) | 110 (35.7) | |
| Systolic pressure | 140 (29) | 140 (27) | 141 (27) | 141 (27) | 0.676 |
| BMI, kg/m2 | 26.9 (4.8) | 28.5 (6.2) | 25.8 (4.7) | 26.8 (5.2) | **<0.001** |
| Triglycerides, mmol/L | 1.3 (0.7) | 1.4 (0.9) | 1.2 (0.7) | 1.3 (0.7) | 0.01 |
| Total cholesterol, mmol/L | 5.5 (1.5) | 5.2 (1.5) | 5.5 (1.4) | 5.1 (1.5) | **<0.001** |
| HDL cholesterol, mmol/L | 1.4 (0.6) | 1.3 (0.5) | 1.4 (0.6) | 1.4 (0.5) | **<0.001** |
| History of comorbidity,% | | | | | |
| Stroke | 30 (1.0) | 5 (2.0) | 3 (1.0) | 7 (2.3) | 0.095* |
| Diabetes mellitus | 233 (7.6) | 20 (8.2) | 28 (9.3) | 31 (10.2) | 0.359 |
| Depression symptom | 244 (7.9) | 15 (6.1) | 26 (8.6) | 34 (11.1) | 0.153 |
| Atrial fibrillation | 132 (4.3) | 31 (12.4) | 19 (6.3) | 30 (9.7) | **<0.001** |
| Heart failure | 44 (1.4) | 17 (6.8) | 5 (1.6) | 25 (8.1) | **<0.001** |
| Coronary heart disease | 224 (7.3) | 33 (13.3) | 29 (9.5) | 42 (13.6) | **<0.001** |
| *APOE*, % | | | | | |
| without APOE-ε4 allele | 2074 (72.1) | 177 (77.6) | 200 (71.4) | 203 (71.5) | 0.299 |
| with APOE-ε4 heterozygotes | 732 (25.5) | 50 (21.9) | 71 (25.4) | 76 (26.8) | |
| with APOE-ε4 homozygotes | 70 (2.4) | 1 (0.4) | 9 (3.2) | 5 (1.8) | |
| Medications, % | | | | | |
| Antihypertensives | 18 (0.6) | 5 (2.0) | 3 (1.0) | 3 (1.0) | 0.073 |
| Diuretics | 401 (13.0) | 63 (25.3) | 27 (8.9) | 63 (20.4) | **<0.001** |
| Vasoprotectives | 4 (0.1) | 0 (0.0) | 0 (0.0) | 1 (0.3) | 0.648 |
| Betablocking agents | 619 (20.1) | 82 (32.9) | 72 (23.8) | 92 (29.9) | **<0.001** |
| Calcium blockers | 229 (7.4) | 27 (10.8) | 29 (9.6) | 39 (12.7) | **0.004** |
| ACE-inhibitors | 765 (24.9) | 78 (31.3) | 72 (23.8) | 92 (29.9) | **0.035** |
| Serum lipid reducing agents | 885 (28.8) | 87 (34.9) | 90 (29.7) | 114 (37.0) | 0.006 |
| Statins | 792 (25.7) | 79 (31.7) | 78 (25.7) | 108 (35.1) | **0.001** |
| FEV1/FVC (%) | 78.7 (6.5) | 76.4 (7.0) | 67.4 (3.5) | 61.8 (10.1) | **<0.001** |

| | | | | | |
|---|---|---|---|---|---|
| FEV1% predicted | 103.4 (18.7) | 73.9 (10.8) | 91.3 (13.7) | 67.0 (16.0) | **<0.001** |
| FVC% predicted | 101.5 (17.9) | 72.2 (12.0) | 106.0 (15.9) | 81.4 (15.0) | **<0.001** |
| Microbleeds, % | 636 (21.0) | 67 (27.0) | 64 (21.0) | 84 (27.0) | **<0.001** |
| Lacunar infarcts, % | 243 (7.9) | 30 (12.0) | 31 (10.2) | 51 (16.6) | **<0.001** |
| Cortical infarcts, % | 95 (3.1) | 12 (4.8) | 16 (5.3) | 17 (5.5) | **0.03** |

Definition of abbreviations: *APOE* = apolipoprotein E; BMI=Body Mass Index; COPD = Chronic Obstructive Pulmonary Disease; $FEV_1$ = Forced Expiratory Volume in one second; FVC = Forced Vital Capacity; HDL = High-density lipoprotein; PRISm = Preserved Ratio Impaired Spirometry. Data represent original data without imputed values. Missing values were present for education attainment (1.0%), systolic blood pressure (2.4%), BMI (1.0%), triglyceride (1.4%), total cholesterol (1.4%), HDL cholesterol (1.4%), history of diabetes (1.1%), depressive symptom (0.4%), and medications, including antihypertensives (0.1%), diuretics (0.1%), vasoprotectives (0.1%), beta-blocking agents (0.1%), calcium blockers (0.1%), ACE-inhibitors (0.1%), serum lipid reducing agents (0.1%) and statins (0.1%).

p-values are for comparing differences among participants with different lung function

*Fisher test

Table 2 The association between lung function and individuals cognition tests presented for cross-sectional (A) and longitudinal analyses (B)

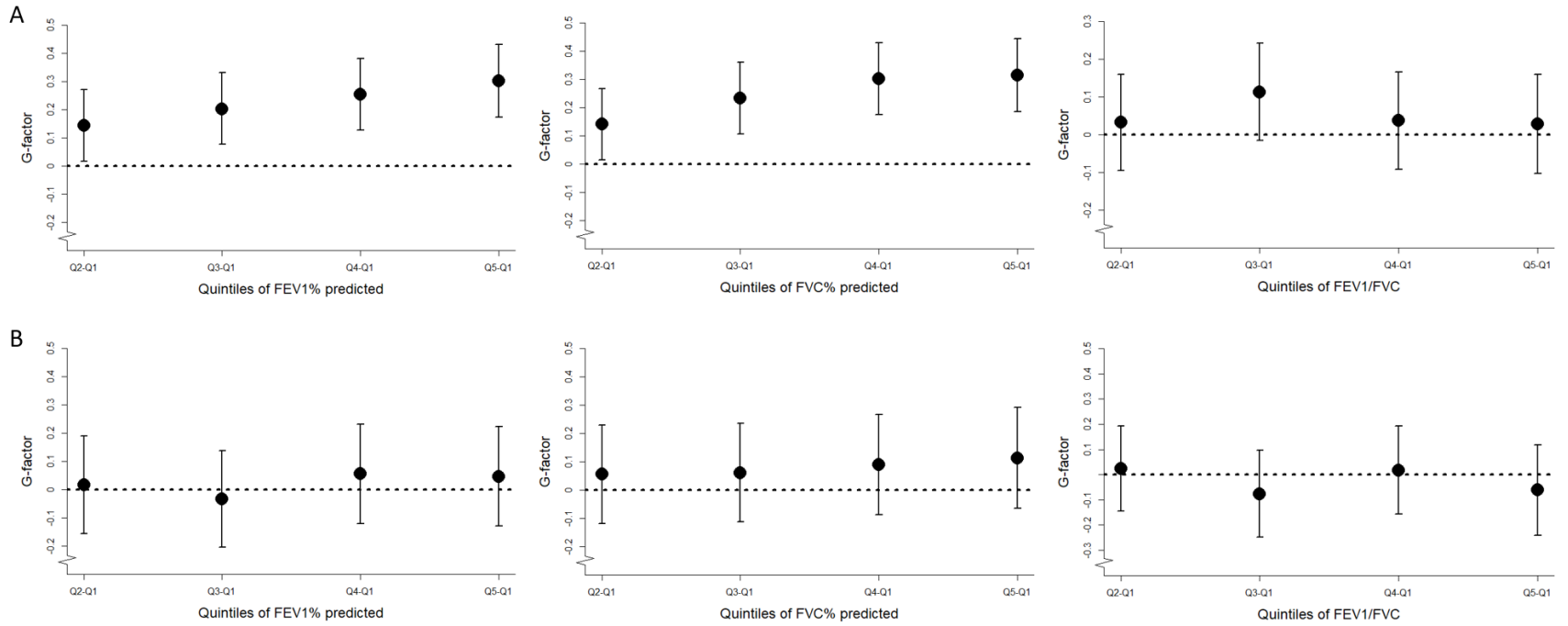
| | | | Cross-sectional analyses (A) | | |
|---|---|---|---|---|---|
| | Number of participants | Normal | PRISm | COPD GOLD1 | COPD GOLD2-4 |
| G-factor | n=3134 | Reference | -0.27 (-0.43;-0.11)* | -0.08 (-0.23;0.06) | -0.22 (-0.37;-0.06)* |
| LDST, z-score | n=3685 | Reference | -0.26 (-0.42;-0.09)* | -0.02 (-0.17;0.13) | -0.18 (-0.33;-0.03)* |
| Stroop test1, z-score | n=3688 | Reference | 0.13 (-0.05;0.30) | -0.01 (-0.16;0.15) | 0.14 (-0.02;0.30) |
| Stroop test2, z-score | n=3688 | Reference | 0.16 (-0.02;0.34) | 0.00 (-0.16;0.16) | 0.25 (0.08;0.42) |
| Stroop test3, z-score | n=3679 | Reference | 0.21 (0.03;0.39)* | 0.05 (-0.11;0.21) | 0.11 (-0.05;0.27) |
| WFT, z-score | n=3743 | Reference | -0.17 (-0.34;0.01) | -0.01 (-0.16;0.15) | -0.14 (-0.31;0.02) |
| WLTdel, z-score | n=3575 | Reference | -0.08 (-0.26;0.09) | -0.08 (-0.24;0.08) | -0.21 (-0.38;-0.05)* |
| WLTimm, z-score | n=3574 | Reference | -0.12 (-0.29;0.06) | 0.05 (-0.10;0.21) | -0.17 (-0.33;-0.01)* |
| WLTrecog, z-score | n=3646 | Reference | -0.10 (-0.28;0.09) | -0.02 (-0.19;0.15) | -0.16 (-0.33;0.01) |
| PPB test, z-score | n=3395 | Reference | -0.17 (-0.34;-0.01)* | -0.10 (-0.25;0.05) | -0.18 (-0.33;-0.03)* |
| | | | Longitudinal analyses (B) | | |
| | Number of participants | Normal | PRISm | COPD GOLD1 | COPD GOLD2-4 |
| G-factor | n=878 | Reference | 0.01 (-0.24;0.26) | 0.15 (-0.04;0.34) | 0.06 (-0.16;0.26) |
| LDST, z-score | n=1225 | Reference | -0.06 (-0.29;0.16) | 0.10 (-0.09;0.29) | -0.05 (-0.26;0.15) |
| Stroop test1, z-score | n=1220 | Reference | -0.13 (-0.39;0.12) | -0.02 (-0.23;0.19) | 0.02 (-0.20;0.24) |
| Stroop test2, z-score | n=1219 | Reference | -0.09 (-0.33;0.15) | -0.01 (-0.21;0.19) | 0.06 (-0.15;0.27) |
| Stroop test3, z-score | n=1210 | Reference | -0.11 (-0.35;0.14) | -0.01 (-0.21;0.20) | 0.05 (-0.18;0.27) |
| WFT, z-score | n=1266 | Reference | -0.06 (-0.34;0.22) | 0.01 (-0.22;0.24) | 0.04 (-0.20;0.28) |
| WLTdel, z-score | n=1221 | Reference | -0.13 (-0.41;0.15) | 0.09 (-0.15;0.32) | 0.06 (-0.19;0.31) |
| WLTimm, z-score | n=1230 | Reference | -0.04 (-0.31;0.24) | 0.18 (-0.06;0.41) | -0.07 (-0.31;0.18) |
| WLTrecog, z-score | n=1209 | Reference | 0.04 (-0.27;0.34) | 0.00 (-0.26;0.25) | 0.06 (-0.22;0.33) |
| PPB test, z-score | n=1118 | Reference | 0.08 (-0.19;0.35) | 0.03 (-0.18;0.25) | -0.03 (-0.26;0.21) |

Definition of abbreviations: *APOE* = apolipoprotein E; BMI=Body Mass Index; COPD = Chronic Obstructive Pulmonary Disease; $FEV_1$ = Forced Expiratory Volume in one second; FVC = Forced Vital Capacity; G-factor = principle component scores of cognition tests; LDST = Letter-Digit Substitution test; HDL = High-density lipoprotein; PRISm = Preserved Ratio Impaired Spirometry; PPB test = Purdue Pegboard test; WFT = Word Fluency test; WLTdel = Word learning test, delayed recall; WLTimm = Word learning test, immediate recall; WLTrecog = Word learning test, recognition. The table presents estimated mean difference (95% confidence interval) in cognitive scores after adjustment for covariates.

A) Represents cross-sectional association between spirometry tests and cognitive tests; B) Represents the longitudinal association between spirometry tests and cognitive tests. Cross-sectional analysis is adjusted for age, sex, education, smoking status, BMI, systolic blood pressure, serum lipids (triglycerides, total cholesterol, HDL cholesterol) , history of comorbidities (stroke, diabetes, depressive symptom, atrial fibrillation, heart failure, coronary heart disease), medications (antihypertensives, diuretics, vasoprotectives, beta-blocking agents, calcium blockers, ACE-inhibitors, serum lipid reducing agents and statins), and *APOE* genotype. Longitudinal analysis is based on cross-sectional model plus time between two cognitive tests and test scores at baseline.

*Statistically significant results

Figure 2. The association between lung function parameters with the G-factor of cognition presented for cross-sectional (A) and longitudinal (B) analyses

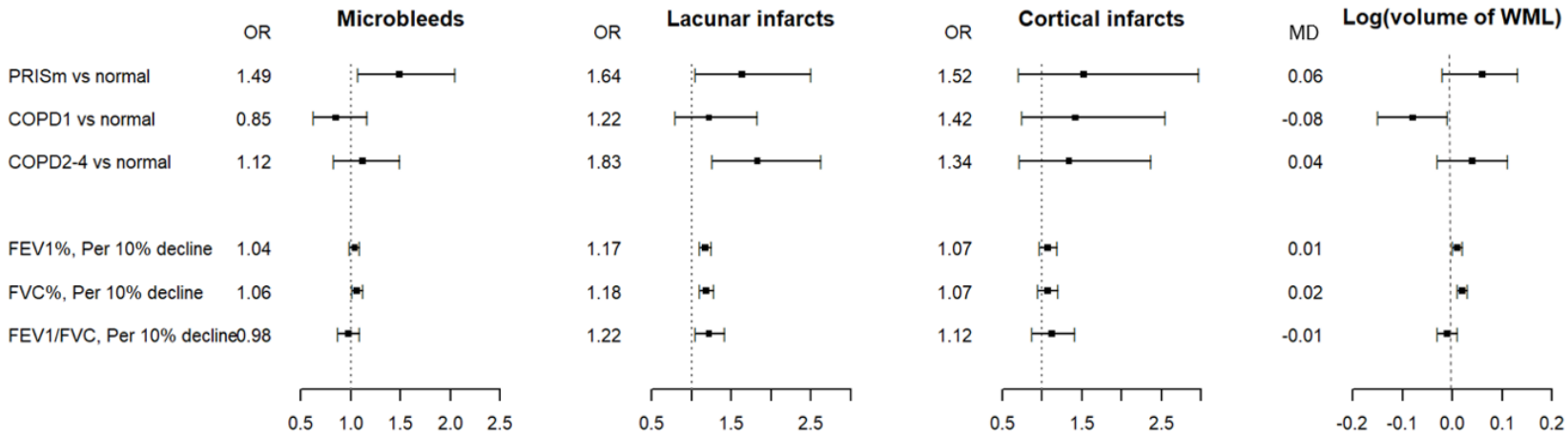



Definition of abbreviations: BMI = Body mass index; $FEV_1$ = Forced Expiratory Volume in one second; FVC = Forced Vital Capacity; G-factor = principle component scores of cognition tests; HDL = High-density lipoprotein; LDL = Low-density lipoprotein.

A) Represents cross-sectional association between spirometry tests and G-factor of cognitive tests; B) Represents the longitudinal association between spirometry tests and G-factor of cognitive tests. The figures show difference of G-factor between subgroups of higher quintile of spirometry parameters and those of the lowest (reference) quintile. Higher G-factor indicate better cognition performance. $Model_A$ with adjustment for age, sex, education, smoking status, BMI, systolic blood pressure, serum lipids (triglycerides, total cholesterol, HDL cholesterol), history of comorbidities (stroke, diabetes, depressive symptom, atrial fibrillation, heart failure,

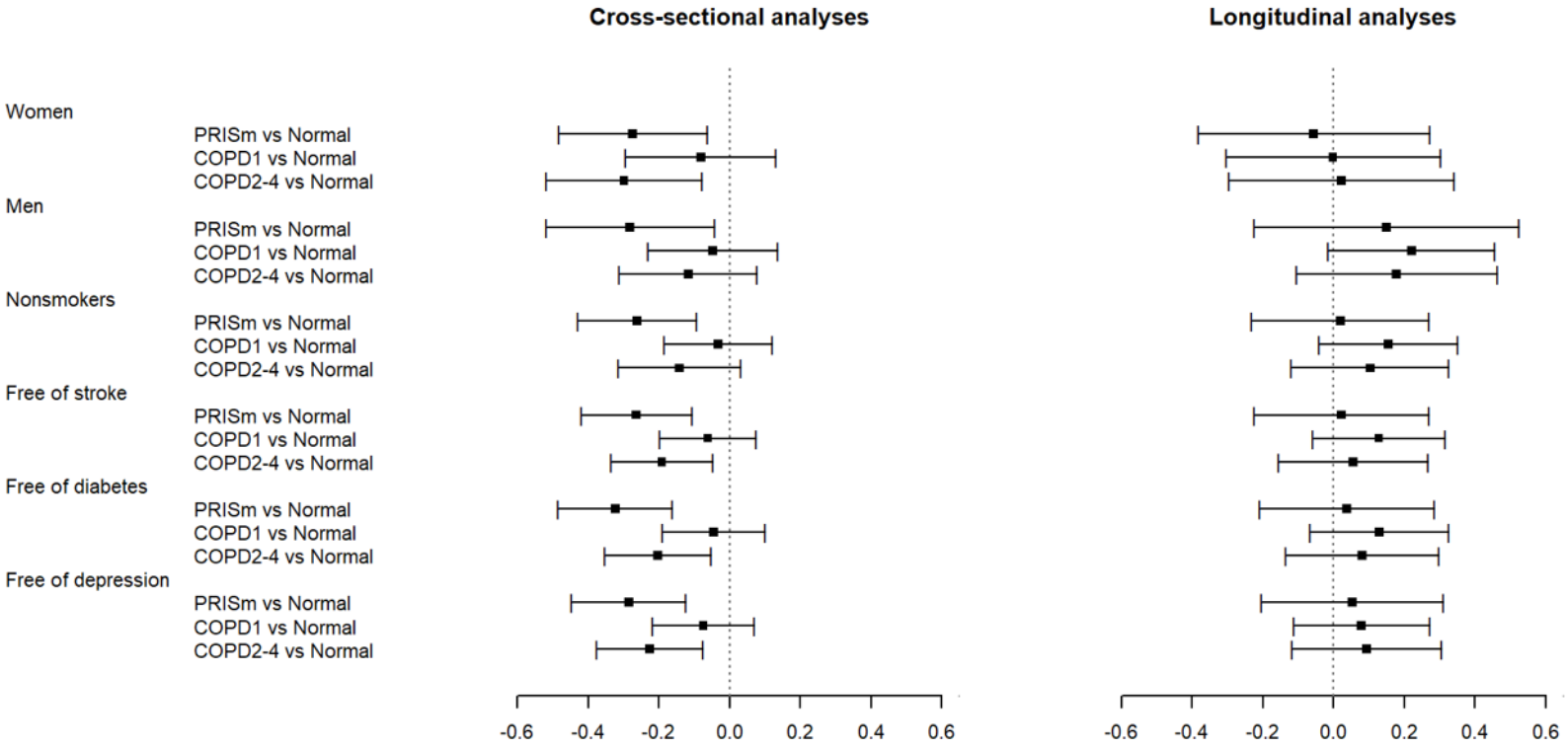
coronary heart disease), medications (antihypertensives, diuretics, vasoprotectives, beta-blocking agents, calcium blockers, ACE-inhibitors, serum lipid reducing agents and statins), and *APOE* genotype; $Model_B$ = $Model_A$ plus time between two cognitive tests and test scores (G-factor) at baseline.

Figure 3. Cross-sectional analysis for vascular brain lesions among participants with different lung function




Definition of abbreviations: BMI = Body mass index; COPD = Chronic Obstructive Pulmonary Disease; $FEV_1$ = Forced Expiratory Volume in one second; FVC = Forced Vital Capacity; MD = Mean Difference; OR = Odds Ratio; PRISm = Preserved Ratio Impaired Spirometry; WML = White Matter Lesion. Odds ratios and mean difference were attained using logistic regression models and linear regression model adjusted for age, sex, education, smoking status, BMI, systolic blood pressure, serum lipids (triglycerides, total cholesterol, HDL cholesterol), history of comorbidities (stroke, diabetes, depressive symptom, atrial fibrillation, heart failure, coronary heart disease), medications (antihypertensives, diuretics, vasoprotectives, beta-blocking agents, calcium blockers, ACE-inhibitors, serum lipid reducing agents and statins), and *APOE* genotype.

S.Figure 1. Mean difference of G-factor between participants with lung function impairment and reference group in cross-sectional (A) and longitudinal analyses (B)




Definition of abbreviations: COPD = Chronic Obstructive Pulmonary Disease; G-factor = principle component scores of cognition tests; HDL = High-density lipoprotein; PRISm = Preserved Ratio Impaired Spirometry. Spirometry test in relation to G-factor of cognition tests in cross-sectional analyses at baseline. Lower G-factor indicates worse cognition performance. A) Represents mean difference of G-factor in cross-sectional analyses; B) Represents mean difference of G-factor in longitudinal analyses.

S. Table1 Representativeness of the study population for longitudinal data analyses

| | Without follow-up | Follow-up | P Value |
|---|---|---|---|
| n (%) | 1975 (52.1) | 1815 (47.9) | - |
| Age, yr | 62.3 (7.5) | 74.2 (8.3) | <**0.001** |
| Female, % | 1070 (54.2) | 989 (54.5) | 0.872 |
| Education level | | | |
| Primary education | 158 (8.0) | 120 (6.7) | <**0.001** |
| lower education | 670 (34.0) | 751 (42.1) | |
| Intermediate education | 565 (28.7) | 588 (33.0) | |
| Higher education | 576 (29.3) | 324 (18.2) | |
| Smoking status, % | | | |
| Never | 678 (34.3) | 620 (34.2) | <**0.001** |
| Former | 982 (49.7) | 1019 (56.1) | |
| Current | 315 (15.9) | 176 (9.7) | |
| Systolic pressure | 132 (24) | 148 (25) | <**0.001** |
| BMI, kg/m2 | 26.9 (5.1) | 27.0 (4.8) | 0.515 |
| Triglycerides, mmol/L | 1.3 (0.8) | 1.3 (0.7) | 0.812 |
| Total cholesterol, mmol/L | 5.6 (1.5) | 5.4 (1.6) | <**0.001** |
| HDL cholesterol, mmol/L | 1.4 (0.6) | 1.4 (0.5) | 0.411 |
| History of comorbidity,% | | | |
| Stroke | 23 (1.2) | 6 (0.3) | **0.004** |
| Diabetes mellitus | 160 (8.1) | 134 (7.5) | 0.345 |
| Depression symptom | 150 (7.6) | 152 (8.4) | 0.744 |
| Atrial fibrillation | 68 (3.4) | 135 (7.4) | <**0.001** |
| Heart failure | 15 (0.8) | 73 (4.0) | <**0.001** |
| Coronary heart disease | 115 (5.8) | 198 (10.9) | <**0.001** |
| *APOE*, % | | | |
| without APOE-ε4 allele | 1265 (69.9) | 1293 (75.5) | <**0.001** |
| with APOE-ε4 heterozygotes | 498 (27.5) | 385 (22.5) | |
| with APOE-ε4 homozygotes | 47 (2.6) | 34 (2.0) | |
| Medications, % | | | |
| Antihypertensives | 18 (0.9) | 8 (0.4) | 0.118 |
| Diuretics | 209 (10.6) | 311 (17.1) | <**0.001** |
| Vasoprotectives | 2 (0.1) | 3 (0.2) | 0.676* |
| Betablocking agents | 321 (16.3) | 492 (27.1) | <**0.001** |
| Calcium blockers | 115 (5.8) | 183 (10.1) | <**0.001** |
| ACE-inhibitors | 398 (20.2) | 553 (30.5) | <**0.001** |
| Serum lipid reducing agents | 538 (27.3) | 574 (31.6) | **0.004** |

| | | | |
|---|---|---|---|
| Statins | 435 (22.1) | 562 (31.0) | <**0.001** |
| Spirometry,% | | | |
| Normal | 1572 (79.6) | 1405 (77.4) | **0.002** |
| PRISm | 128 (6.5) | 109 (6.0) | |
| COPD1 | 155 (7.8) | 130 (7.2) | |
| COPD2-4 | 120 (6.1) | 171 (9.4) | |
| $FEV_1$/FVC (%) | 77.8 (8.0) | 77.0 (8.6) | <**0.001** |
| $FEV_1$% predicted | 99.6 (20.9) | 99.7 (24.2) | 0.538 |
| FVC% predicted | 100.0 (18.6) | 98.7 (21.4) | **0.020** |

Definition of abbreviations: *APOE* = apolipoprotein E; BMI = Body Mass Index; COPD = Chronic Obstructive Pulmonary Disease; $FEV_1$ = Forced Expiratory Volume in one second; FVC = Forced Vital Capacity; LDST = Letter-Digit Substitution test; HDL = High-density lipoprotein; PRISm = Preserved Ratio Impaired Spirometry; PPB test = Purdue Pegboard test; WFT = Word Fluency test; WLTdel = Word learning test, delayed recall; WLTimm = Word learning test, immediate recall; WLTrecog = Word learning test, recognition.

p-values are for comparing differences between participants within and lost to follow-up

*Fisher test

S. Table2 Binomial logistic regression analyses for effect of lung function on cognitive decline

| | Odds ratios (ORs) for cognitive decline | | | | |
|---|---|---|---|---|---|
| | Number of participants | Normal | PRISm | COPD GOLD1 | COPD GOLD2-4 |
| LDST | n=1225 | Reference | 0.99 (0.56;1.75) | 0.75 (0.45;1.22) | 1.55 (0.93;2.61) |
| Stroop test1 | n=1220 | Reference | 0.80 (0.44;1.44) | 1.03 (0.63;1.70) | 1.03 (0.61;1.73) |
| Stroop test2 | n=1219 | Reference | 0.65 (0.35;1.19) | 0.94 (0.57;1.55) | 1.13 (0.67;1.92) |
| Stroop test3 | n=1210 | Reference | 0.77 (0.39;1.46) | 0.94 (0.54;1.59) | 1.01 (0.58;1.76) |
| WFT | n=1266 | Reference | 1.28 (0.72;2.31) | 0.95 (0.59;1.55) | 0.86 (0.53;1.42) |
| WLTdel | n=1221 | Reference | 1.00 (0.55;1.81) | 0.62 (0.36;1.05) | 1.00 (0.58;1.71) |
| WLTimm | n=1230 | Reference | 1.17 (0.65;2.13) | 0.82 (0.49;1.37) | 1.04 (0.61;1.77) |
| WLTrecog | n=1209 | Reference | 0.64 (0.33;1.19) | 1.00 (0.58;1.68) | 0.96 (0.55;1.64) |
| PPB test | n=1118 | Reference | 1.01 (0.52;1.97) | 1.03 (0.61;1.74) | 0.88 (0.50;1.57) |

Definition of abbreviations: BMI = Body mass index; COPD = Chronic Obstructive Pulmonary Disease; LDST = Letter-Digit Substitution Task; PRISm = Preserved Ratio Impaired Spirometry; PPB test = Purdue Pegboard test; Word Fluency Task = WFT; WLTdel = 15-Word Learning Test of delayed recall; WLTimm = 15-Word Learning Test of immediate recall; WLTrecog = 15-Word Learning Test of recognition. The table presents odds ratio (OR) (95% confidence interval) in cognitive scores after adjustment for covariates. For some cognitive tests, including LDST, WFT, WLTdel, WLTimm, WLTrecog and PPB test, cognitive decline was defined as a drop in following-up scores of more than mean difference between baseline and following-up tests per year. On the contrary, cognitive decline was defined as an increase in following-up scores of more than mean difference in Stroop tests. Analyses are adjusted for age, sex, education, smoking status, BMI, systolic blood pressure, serum lipids (triglycerides, total cholesterol, HDL cholesterol) , history of comorbidities (stroke, diabetes, depressive symptom, atrial fibrillation, heart failure, coronary heart disease), medications

(antihypertensives, diuretics, vasoprotectives, beta-blocking agents, calcium blockers, ACE-inhibitors, serum lipid reducing agents and statins), and *APOE* genotype.

# Chapter 3 Fatty Liver Disease and Neurodegeneration

# Chapter 3.1 Association of Nonalcoholic Fatty Liver Disease and Fibrosis With Incident Dementia and Cognition: The Rotterdam Study

**Abstract**

**Introduction:** We investigated the association of NAFLD and fibrosis with incident-dementia and cognition among the elderly.

**Methods:** We included non-dementia participants at baseline with available fatty liver index (FLI) (set 1; visit 1997-2002; n=3,975; FU=15.5 years) or with abdominal ultrasound (set 2; visit 2009-2014; n=4,577; FU=5.7 years) or liver stiffness (set 3; visit 2009-2014; n=3,300; FU=5.6 years). Cox-regression was used to quantify associations for NAFLD or liver fibrosis with incident-dementia and logistic regression for NAFLD and cognitive function.

**Results:** NAFLD and fibrosis were consistently not associated with increased risk of incident dementia. Interestingly, NAFLD was associated with a significantly decreased risk for incident-dementia until five years after FLI-assessment. Moreover, NAFLD was not associated with worse cognitive function.

**Discussion:** NAFLD and fibrosis were not associated with increased risk for incident-dementia. In contrast, NAFLD was even protective in the first five years of follow-up, hinting towards NAFLD regression before dementia onset.

**Keywords**

NAFLD; fibrosis; liver stiffness; cognition; dementia; epidemiology; general population; longitudinal analysis

## Introduction

Non-alcoholic Fatty Liver Disease (NAFLD) is increasingly common and affects >25% of the global population [136]. It has become one of the most prevalent chronic liver diseases, ranging from simple fat accumulation to liver cirrhosis [137]. In addition, recent studies indicate that NAFLD is associated with kidney dysfunction [138, 139], cardiovascular disease [140] and extra-hepatic malignancies such as colon and stomach cancer [141, 142]. However, its link with neurodegenerative conditions, such as dementia or cognition impairment remains unclear.

As a metabolic disease, NAFLD has several risk factors in common with dementia, for example, insulin resistance, hypertension, obesity, physical inactivity and dyslipidemia [143]. Accumulating evidence also suggests a direct association of NAFLD with brain structural changes via the so-called liver-brain axis [144-146]. This might link NAFLD to dementia, driven by the following mechanisms: 1) inflammation due to liver fat may activate microglial cells resulting in elevated expression of inflammatory cytokines in the brain [147]; 2) increased brain insulin resistance in patients with NAFLD may cause oxidative stress, excessive free fatty acids and brain mitochondrial disorders [148]; 3) cerebrovascular and hemodynamic disturbances provoked by a prothrombotic state [143]. Despite this growing evidence for a liver-brain axis, current available studies reported no effects of NAFLD on dementia [149, 150] or only in frail NAFLD participants with fibrosis [151]. However, some other studies indicated that cognitive impairment was more common in patients with NAFLD [152] or fibrosis [153], which might indicate a potential association with dementia and NAFLD.

The majority of those studies are, however, cross-sectional, had limited follow-up or had a small sample size. Moreover, some studies lacked abdominal imaging to determine steatosis and transient elastography was often not available to assess fibrosis. Given these limitations and the

inconsistent results, the impact of NAFLD on dementia remains unclear. Therefore, we aim to study the associations of NAFLD and fibrosis with incident dementia and cognitive function in a well-defined, prospective cohort with available ultrasound and transient elastography data. A defining feature of our study is the use of different measures of NAFLD using various modalities that together provide a comprehensive assessment of liver function.

## Methods

### Participants

This study was conducted within the Rotterdam Study, a prospective ongoing cohort that started in 1990. Participants aged at least 45 years from a suburb in Rotterdam were included to investigate chronic diseases in the general population [45]. Study visits comprised a home interview and various physical examinations at the research center and were repeated every four to six years. In this study, we included three different sets (FIGURE 1) in which we assessed the impact of NAFLD or fibrosis on the risk of incident dementia several ways. Set 1 comprised of participants in whom we had available fatty liver index (FLI) to determine NAFLD, measured between 1997 and 2002. Set 2 comprised of participants visiting the study center between 2009 and 2014 in whom we had abdominal ultrasound performed to assess NAFLD. Set 3 is a subset of set 2, and comprises participants that also underwent liver stiffness measurement to assess fibrosis. Sets 2 and 3 were also used to investigate the association with cognition cross-sectionally.

Exclusion criteria were: 1) Prevalent dementia; 2) Lack of follow-up; 3) Missing dementia data; 4) Secondary causes for steatosis or missing alcohol data. These secondary causes were steatosis-inducing drug use (i.e. amiodarone, corticosteroids and methotrexate), viral hepatitis or excessive alcohol consumption (>20 grams/day for female or >30 grams/day for male) assessed by food frequency questionnaire (FFQ) or alcohol interview [154]. In addition, for set 3, participants with invalid liver stiffness measurements were also excluded. (Supplementary table 1).

### Steatosis assessment

NAFLD was defined as the presence of FLI $\geq$ 60 (set 1) or hepatic steatosis based on abdominal ultrasound (set 2) in the absence of secondary causes for steatosis. FLI was calculated with the following algorithm: FLI = ($e^{0.953}$*$\log_e$ (triglycerides) + 0.139*BMI + 0.718*$\log_e$ (GGT) +

0.053*waist circumference - 15.745) / (1 + $e^{0.953}$*$\log_e$ (triglycerides) + 0.139*BMI + 0.718*loge (GGT) + 0.053*waist circumference - 15.745) x 100, where triglycerides were measured in mg/dL, GGT in U/L, waist circumference in cm, and BMI in kg/$m^2$. Participants were categorized according to their FLI score as no NAFLD for FLI <30 and NAFLD for FLI ≥ 60 [155]. Steatosis based on abdominal ultrasound was defined as hyperechoic liver parenchyma compared to the spleen or kidney according to the protocol of Hamaguchi et al [156]. Abdominal ultrasound was performed by a single certified and experienced sonographer (PVW) on a Hitachi Hi Vision 900.

**Fibrosis assessment**

Liver stiffness was assessed using transient elastography (FibroScan, EchoSens, Paris, France). At least ten measurements were obtained through either M or XL probe according to the device's instructions. Final measurements >7.1 kilopascal (kPa) with an interquartile range > 30% were considered unreliable and discarded, according to the Boursier criteria [157]. Liver fibrosis was defined as liver stiffness measurement (LSM) ≥ 8.0 kPa [158].

**Dementia assessment**

Dementia assessment was performed at baseline and subsequent center visits with the Mini-Mental State Examination and the Geriatric Mental Schedule [49]. Those with a Mini-Mental State Examination score <26 or Geriatric Mental Schedule score >0 underwent further investigation including Cambridge Examination for Mental Disorders of the Elderly. Moreover, diagnosis of dementia by other health care professionals was available through electronic linkage of the study database with medical records from general practitioners and the regional institute for outpatient mental health care. An adjudication panel led by a consultant neurologist established the final diagnosis according to standard criteria for dementia (Diagnostic and Statistical Manual of Mental

Disorder, Third Edition-Revised: DSM-III-R). Follow-up was complete until 1st January 2018. Within this period, participants were followed until the date of dementia, death, loss to follow-up or 1st of January 2018, whichever came first.

**Cognitive testing**

Participants underwent several neuropsychological tests during the study visit, this includes the Stroop tests (trial 1 for word naming, trial 2 for color naming and trial 3 for color naming and matching with color word), the Letter-Digit Substitution Task (LDST), the Word Fluency Task (WFT), a 15-Word Learning Test with immediate (WLTimm) and delayed recall (WLTdel), and Purdue Pegboard test (PPB test), which are described in supplementary table 1. These test results were transformed into a Z-score, this reflects the number of standard deviations the test results were below or above the mean score. To assess the overall cognitive function, a general cognitive factor (G-factor) was calculated using only the LDST, WFT, WLTdel tests and the trial 3 of Stroop test, to prevent distortion by highly correlated tasks [93]. Detailed information was presented in Supplementary table 2.

**Covariates**

Demographic and physiological information included age, sex, education level (lower education, intermediate education, higher education), smoking status (never, former, current), alcohol intake (units/day), body mass index (BMI, $kg/m^2$), alanine aminotransferase (ALT, U/L) and chronic comorbid conditions (diabetes, hypertension and stroke) [45]. Apolipoprotein E (*APOE*) genotype was determined using a PCR and a bi-allelic TaqMan assay (rs7412 and rs429358) on labelled DNA samples. *APOE*-ε4 allele represented carrier of one or two ε4 alleles.

**Statistical analysis**

Baseline characteristics are described for the overall population in all three sets. Data are expressed as mean ± standard deviation (SD) or as median (with 25$^{th}$ and 75$^{th}$ percentile [P25-P75]). For time-to-event analyses, we assessed the associations between of NAFLD and liver stiffness with the risk of incident dementia using Cox proportional-hazards regression analyses. Baseline was defined as date of blood test (for FLI) or abdominal ultrasound and follow-up ended at the diagnosis of dementia, death, loss to follow-up, or 1$^{st}$ January 2018. Model 1 was adjusted for APOE phenotype, age, sex and education. Model 2 was in addition adjusted for alcohol, smoking, stroke, hypertension, diabetes and cholesterol. Model 3 was in addition adjusted for BMI. Covariates above were selected based on previous literature, clinical relevance, and data availability [37, 159].

Next, we determined the cross-sectional association of NAFLD or fibrosis with cognitive function using linear regression analyses and Tukey all-pair comparisons method based on ANOVA models. We calculated the differences of the individual cognitive tests and G-factor for participants with NAFLD compared to those without NAFLD and for fibrosis compared to no fibrosis. Results were adjusted for age, sex, education level, smoking status, BMI, cholesterol, triglycerides, hypertension, stroke, diabetes, depression and APOE genotypes.

A p-value of $<0.05$ was considered statistically significant. All analysis were performed using R version 4.0.4 (Foundation for Statistical Computing, Vienna, Austria).

## Results

### Baseline characteristics of the overall population

There were 3,975 participants with available NAFLD data based on FLI included in set 1, 4,577 participants with available ultrasound to assess NAFLD in set 2, and 3,300 participants with available liver stiffness measurement to assess fibrosis in set 3, exclusions are described in supplementary table 2. Participants from the different sets had a similar mean age (around 70 years), BMI (near 27 kg/m2) and approximately 60% of them were women. In set 1, 1293 (32.5%) participants had NAFLD (FLI ≥ 60), and in set 2, 1586 (34.7%), which was based on abdominal ultrasound. In set 3 the median liver stiffness was 4.8 kPa (P25-P75: 3.8-5.9) and 192 (5.8%) participants had fibrosis. (TABLE 1)

As shown in FIGURE 1, in set 1, 753 (18.9%) participants developed dementia during a median follow-up of 15.5 years. In set 2 the median follow-up was 5.7 years, and 262 (5.7%) participants had incident dementia. In set 3, only 127 (3.8%) had incident dementia with 5.6 years of median follow up. Participants' characteristics stratified by NAFLD status for set 1 and 2 are presented in supplementary table 3.

### NAFLD and fibrosis in relation to incident dementia

The presence of NAFLD (based on FLI ≥ 60, set 1) did not increase the risk of incident dementia (HR: 0.92; 95% confidence interval (CI): 0.69–1.22) in the fully adjusted model. Similarly, no increased risk of dementia could be demonstrated for the presence of NAFLD, based on abdominal ultrasound in set 2. NAFLD was even associated with a significantly decreased risk for incident dementia in model 2 (HR 0.73, 95% CI: 0.54 – 0.98), which was no longer significant after additional adjusting for BMI (HR: 0.84; 95% CI: 0.61–1.16). Consistent with those results, no

association was found for fibrosis (HR: 1.07; 95% CI: 0.58-1.99) or liver stiffness (HR: 1.01 per kPa; 95% CI: 0.92–1.10) with incident dementia in fully adjusted models in set 3 (Table 2). Interestingly, for the first five years of follow-up, participants with NAFLD (FLI ≥ 60, set 1) were at a significantly lower risk of incident dementia (HR: 0.49; 95% CI: 0.25–0.96) in the fully adjusted model, compared to no NAFLD (FLI < 30). With the period of follow-up extending, the protective association between NAFLD and risk of incident dementia disappeared (between 5-10 years, HR: 1.08; 95% CI: 0.62–1.87; above 10 years, HR: 1.25; 95% CI: 0.80–1.96, Table 3). Weight loss prior to abdominal ultrasound since their previous visit (mean time between visits 6.1 years) was more evident among participants that had developed dementia during the follow up, compared to those without incident dementia (mean: -0.37 vs -0.05 kg per year; set 2).

**NAFLD and liver fibrosis in relation to cognitive performance**

Figure 2 presents the association of NAFLD (abdominal ultrasound, set 2) and liver fibrosis (set 3) with cognitive performance. Cross-sectional analyses revealed that NAFLD was not significantly associated with poor performance on global cognition (Mean difference (MD) of Z-score) in G-factor score compared to reference group without NAFLD: 0.032 (95%CI: -0.029;0.092); in fact, better performance of Stroop test 2 was observed in cross-sectional analyses. On the contrary, we found that liver fibrosis was associated with lower global cognition scores (MD compared to reference group without liver fibrosis: –0.172, 95% CI: -0.307;-0.037) and lower scores of LDST and more time to finish Stroop test 1 and 3 (Supplementary table 4).

## Discussion

We investigated the impact of NAFLD on dementia and cognitive function in a large prospective ongoing population-based cohort with up to 15.5 years median follow-up. NAFLD was not associated with an increased risk of incident dementia or impaired cognitive function. In addition, the presence of NAFLD was not associated with impaired cognitive function.

In contrast to the suggested liver-brain axis in previous studies, NAFLD did not increase the risk of incident dementia in this study, regardless of the modality of diagnosis (FLI or ultrasound). We even found NAFLD to be significantly protective for dementia within the first five years after FLI-assessment. Similar trends were seen for the association between ultrasound-based NAFLD and incident dementia during the 5.7 years median follow-up. This points us towards one of the challenges regarding NAFLD and dementia research: the reversibility of NAFLD due to weight loss [160]. Dementia, albeit unintentionally, is also accompanied by weight loss during its preclinical phase [161], which was confirmed by our results. This could induce NAFLD regression, as even minor improvements in body fat have rather large effects on liver fat and hepatic triglycerides [162, 163]. Consequently, weight loss in the years prior to dementia could thus obscure any relation between NAFLD and incident dementia. In our study, the demonstrated protective effect of NAFLD on dementia disappeared after five years. This suggests that if NAFLD is associated with an increased risk for dementia at all, it is a long-term effect, and NAFLD itself might already have disappeared before dementia is diagnosed.

Given the reversibility of NAFLD, exposure duration could be of major importance to comprehend the association between NAFLD and dementia. Individuals with NAFLD can develop permanent liver fibrosis, resulting in higher liver stiffness, based on the duration and severity of NAFLD [164]. Therefore, we assessed the association between fibrosis and liver stiffness with incident dementia

longitudinally. In line with our results for NAFLD, fibrosis and liver stiffness were also not associated with incident dementia, indicating that NAFLD nor severity of NAFLD is associated with increased risk for incident dementia. Considering cognitive impairment as a classic prodromal symptom preceding the onset of dementia [33], we explored the cross-sectional association between NAFLD and cognition. Similarly, we did not find a significant association between NAFLD and impaired cognitive function**.** However, fibrosis was significantly associated with impaired performance on the Stroop Test, Letter-Digit substitution test resulting in lower G-factor score. These tests cover attention and concentration, processing speed and global cognitive function respectively. Further research is required whether this hints towards an association with dementia as well, or is driven by common risk factors (e.g. the presence of diabetes or metabolic syndrome) or accumulation of toxins by impaired liver function.

Given these consistently negative results, we cannot demonstrate an association of NAFLD with dementia or cognitive function within our follow-up duration. This is in line with a recent registry study among over 40.000 participants, which could not link NAFLD and dementia using ICD-10 codes [149]. Moreover, a study with almost 20 years of follow-up could not identify NAFLD as risk factor for incident dementia[150]. However, they reported that histology proven fibrosis improved the prediction of dementia. Fibrosis was also linked to dementia among the frail elderly previously[151]. However, these results need to be interpreted with caution since fibrosis was calculated based on age, which itself is undisputedly associated with dementia.

More literature is available on cognitive function, and in these studies NAFLD has been linked to impaired performance on serial digit learning test [152] and symbol digit substitution test [152], reduced reaction time [152], lower MoCA scores [165, 166], brain volume reduction [144], and reduced brain activity [166]. However, most results were unadjusted or disappeared after adjustment for important

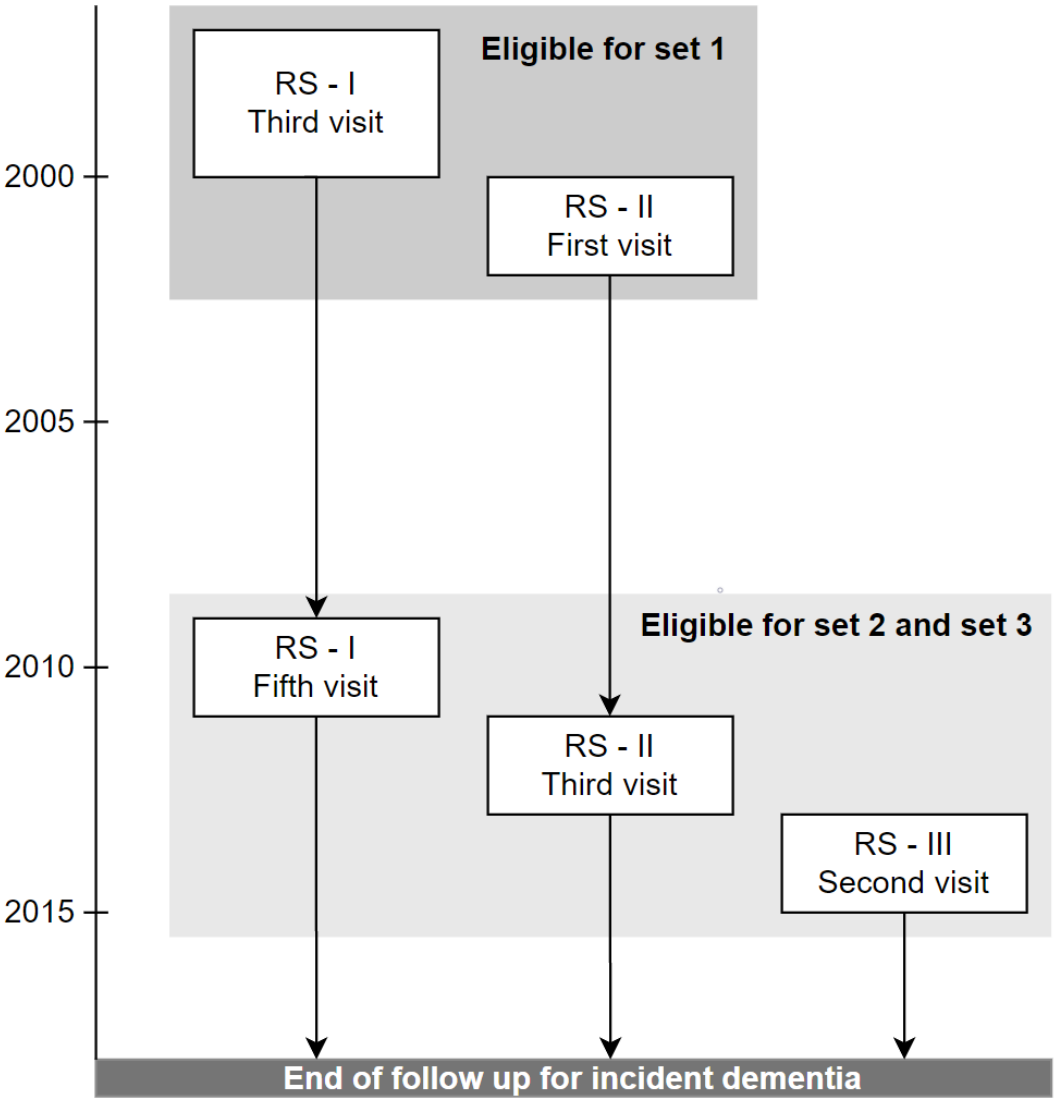
confounders such as age and education level. Moreover, most findings were not replicated and some studies, similar to ours, could not identify any association with NAFLD and cognition [153]. Therefore, the effect of NAFLD on cognitive function seems to be minor, if existing at all.

Although this study had a large sample size and extensive analysis were performed for both incident dementia and cognitive function in relation to NAFLD and fibrosis, the following limitations need mentioning. First, this cohort is almost entirely European, with a mean age of 70 years at baseline. Therefore, our results might not be generalizable to multi-ethnic and younger populations. Second, NAFLD and fibrosis were not based on liver biopsy since that procedure is invasive and subject to potential complications and therefore unethical to perform in a healthy population on this scale. Alternatively, we used abdominal ultrasound in set 2 and FLI in set 1, which correlates strongly with ultrasound diagnosis of NAFLD[167]. Despite fully adjusted models, residual confounding might not be ruled out, as FLI includes BMI. In line with this limitation, NAFLD was only assessed at baseline and no data was available for NAFLD exposure duration. Third, because we had only 192 cases of fibrosis, we might not have found an association with incident dementia. Therefore, the continuous outcome of liver stiffness was also used to explore associations with incident dementia, it should be noted however that this might not reflect only liver injury per se. Fourth, the cross-sectional study design for NAFLD and cognition allows not to study causal relationships for NAFLD on cognition. However, it served as indirect evidence for the absence of associations between NAFLD and dementia, in line with the longitudinal analysis.

## Conclusion

In conclusion, individuals with NAFLD were not at increased risk of dementia among this general elderly population, nor could an association with liver stiffness or fibrosis and dementia be demonstrated. Moreover, NAFLD was associated with a reduced risk of dementia for the first five years after the assessment, suggesting that NAFLD regression is likely before dementia onset, which could be driven by weight loss before dementia onset. As yet, NAFLD may have no clinical implications for dementia awareness. Further studies should focus on NAFLD exposure duration, NAFLD trajectory and risk of dementia with longer follow up durations.

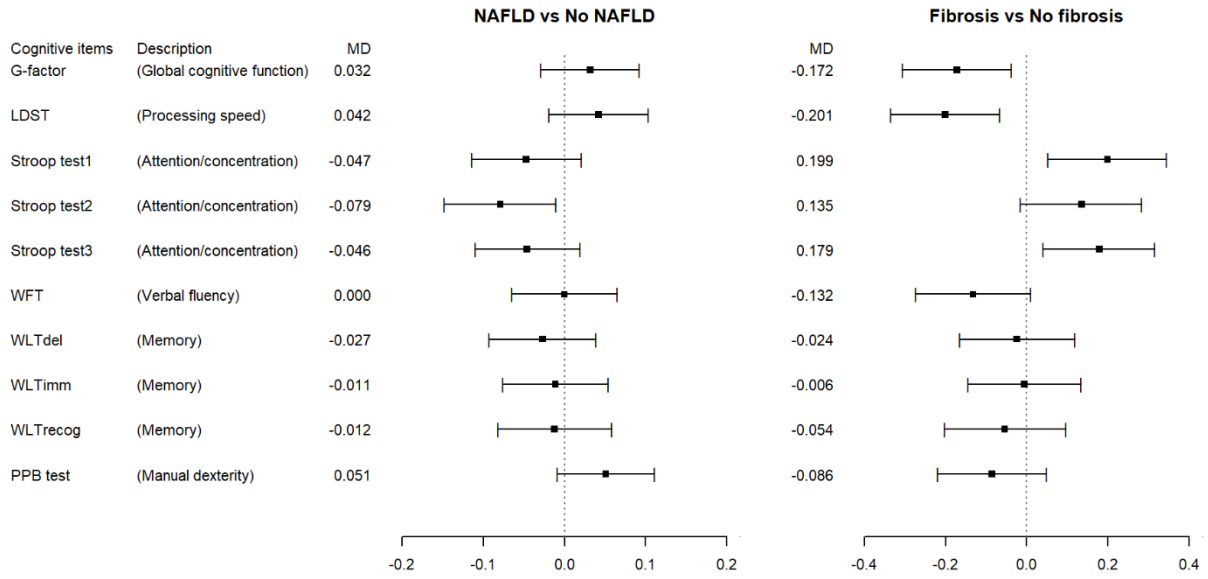
FIGURE 1 Overview of different study sets and key characteristics for investigating the association between NAFLD and fibrosis with dementia and cognitive function.




| | Liver assessment | Inclusions | Follow-up | Events |
|---|---|---|---|---|
| **Set 1** | Fatty Liver Index | 3.975 | 15.5 year | 753 |
| **Set 2** | Abdominal ultrasound | 4.577 | 5.7 year | 262 |
| **Set 3** | Transient elastography | 3.300 | 5.6 year | 127 |

Set 1 and set 2 were used to study associations between NAFLD (either based on FLI or ultrasound) with incident dementia. Set 3 was used to study associations between liver stiffness and fibrosis with incident dementia. Additionally, the impact of NAFLD and fibrosis on cognitive function was studied cross-sectionally in set 2 and set 3.

FIGURE 2 Mean difference of performance on cognitive tests between participants with NAFLD compared to no NAFLD and fibrosis compared to no fibrosis expressed in z-scores.




Presence of NAFLD or fibrosis, in relation to cognition tests in cross-sectional analyses. Higher scores indicate better performance, except for the Stroop tests. Results were obtained from linear regression analyses and Tukey all-pair comparisons method based on ANOVA models. Differences were calculated for the individual cognitive tests and G-factor for participants with NAFLD compared to those without NAFLD and for fibrosis compared to no fibrosis. Results were adjusted for age, sex, education level, smoking status, BMI, cholesterol, triglycerides, hypertension, stroke, diabetes, depression and APOE genotypes. Abbreviations: *APOE*, apolipoprotein E; G-factor, principle component scores of cognition tests; LDST, Letter-Digit Substitution test; MD, Mean difference; PPB test, Purdue Pegboard test; WFT, Word Fluency test; WLTdel, Word learning test, delayed recall; WLTimm, Word learning test, immediate recall; WLTrecog, Word learning test, recognition.

TABLE 1 Baseline characteristics per analysis set

| | **Set 1** | **Set 2** | **Set 3** |
|---|---|---|---|
| | n = 3.975 | n = 4.577 | n = 3.300 |
| **Demographics** | | | |
| Age (years) | 70.0 (8.0) | 69.9 (9.1) | 67.6 (8.4) |
| Female | 2408 (60.6) | 2709 (59.2) | 1892 (57.3) |
| Alcohol consumption | 3068 (77.2) | 3866 (84.5) | 2830 (85.8) |
| Former/current smoking | 2495 (63.1) | 2933 (64.2) | 2081 (63.2) |
| Educational level | | | |
| Low | 2357 (59.8) | 2237 (49.4) | 1517 (46.4) |
| Intermediate | 1129 (28.6) | 1355 (29.9) | 972 (29.7) |
| High | 456 (11.6) | 934 (20.6) | 779 (23.8) |
| **Physical examination** | | | |
| BMI (kg/m$^2$) | 27.0 (4.1) | 27.6 (4.4) | 27.1 (3.9) |
| Enlarged waist circumference* | 1799 (45.3) | 2015 (44.1) | 1356 (41.1) |
| **Comorbidity** | | | |
| Metabolic syndrome | 1983 (50.0) | 1869 (41.6) | 1252 (38.5) |
| Diabetes | 549 (13.8) | 715 (15.8) | 458 (14.0) |
| Stroke | 71 (1.8) | 122 (2.7) | 59 (1.8) |
| Hypertension | 2727 (68.8) | 3374 (73.7) | 2276 (69.0) |
| **Biochemistry / genetics** | | | |
| ALT (U/L) | 20 [16, 25] | 18 [15, 24] | 18 [14, 24] |
| GGT (U/L) | 23 [17, 32] | 23 [17, 33] | 22 [16, 33] |
| Cholesterol (mmol/L) | 5.78 (0.97) | 5.42 (1.11) | 5.48 (1.10) |
| Triglycerides (mmol/L) | 1.34 [1.02, 1.83] | 1.27 [0.98, 1.72] | 1.26 [0.97, 1.70] |
| APOE-ε4 | 1062 (27.8) | 1137 (26.7) | 842 (27.5) |
| **Hepatic comorbidity** | | | |
| NAFLD† | 1293 (32.5) | 1586 (34.7) | 1066 (32.3) |
| Liver stiffness (kPa) | - | 4.8 [3.8, 5.9] | 4.8 [3.8, 5.9] |

Abbreviations: APOE, apolipoprotein E; ALT, alanine transaminase; BMI, body mass index; FLI, fatty liver index; GGT, gamma glutamyl transpeptidase; NAFLD, non-alcoholic fatty liver disease. Data is presented as mean (SD), median [P25-P75] or n and percentage. Baseline characteristics

are presented per set. *Waist circumference > 102 cm for male and > 88 cm for female. †Based on FLI ≥ 60 in set 1 or ultrasound in set 2 and 3.

TABLE 2 Risk of incident dementia for NAFLD and liver stiffness

| | **cases** | **FU*** (year) | **Model 1** | | **Model 2** | | **Model 3** | |
|---|---|---|---|---|---|---|---|---|
| | | | HR | 95% CI | HR | 95% CI | HR | 95% CI |
| **NAFLD (FLI ≥ 60)** | 753/3975 | 15.5 | 0.91 | 0.76 - 1.10 | 0.79 | 0.65 - 0.97 | 0.92 | 0.69 – 1.22 |
| **NAFLD (Ultrasound)** | 262/4577 | 5.7 | 0.87 | 0.66 – 1.15 | 0.73 | 0.54 – 0.98 | 0.84 | 0.61 – 1.16 |
| **Fibrosis**† | 127/3300 | 5.6 | 1.12 | 0.61 – 2.05 | 1.08 | 0.58 – 2.00 | 1.07 | 0.58 – 1.99 |
| **Liver stiffness (kPa)** | 127/3300 | 5.6 | 1.02 | 0.95 – 1.10 | 1.00 | 0.92 – 1.09 | 1.01 | 0.92 – 1.10 |

Abbreviations: APOE, apolipoprotein E; BMI, body mass index; CI, confidence interval; FLI, fatty liver index; FU, follow-up; HR, hazard rate; kPa, kilopascals; NAFLD, non-alcoholic fatty liver disease. Results are given as HR and 95% CI for incident dementia as outcome. Model 1: adjusted for APOE-4, age, sex and education; Model 2 was in addition adjusted for alcohol, smoking, stroke, hypertension, diabetes and cholesterol; Model 3 was in addition adjusted for BMI. NAFLD was either based on FLI ≥ 60 or on hepatic steatosis assessed with abdominal ultrasound and was compared to participants with FLI < 30 or participants without hepatic steatosis.

*Median follow up in years. †Defined as LSM ≥ 8.0 kPa.

TABLE 3 Risk of incident dementia for NAFLD based on fatty liver index per 5 years of follow up

| **Period** | **cases** | **Model 1** | | **Model 2** | | **Model 3** | |
|---|---|---|---|---|---|---|---|
| | | HR | 95% CI | HR | 95% CI | HR | 95% CI |
| **0 – 5 years** | 155/3975 | 0.59 | 0.38 – 0.91 | 0.50 | 0.32 – 0.80 | 0.48 | 0.24 – 0.94 |
| **5 – 10 years** | 194/3472 | 0.85 | 0.59 – 1.21 | 0.78 | 0.54 – 1.14 | 1.10 | 0.63 – 1.91 |
| **> 10 years** | 404/2786 | 1.11 | 0.87 – 1.43 | 0.94 | 0.71 – 1.23 | 1.07 | 0.72 – 1.57 |

Abbreviations: APOE, apolipoprotein E; BMI, body mass index; CI, confidence interval; HR, hazard rate; NAFLD, non-alcoholic fatty liver disease. Model 1 was adjusted for APOE-4, age, sex and education; Model 2 was in addition adjusted for alcohol, smoking, stroke, hypertension, diabetes and cholesterol; Model 3 was in addition adjusted for BMI. NAFLD was based on FLI ≥ 60 and compared to FLI < 30.

Supplementary table 1: details of the cognitive tests used in cross-sectional analysis

| | |
|---|---|
| **Letter Digit Substitution Task (LDST)** | Processing speed was measured with the LDST. After providing an example with the correct combinations, participants were asked to make as many letter-digit combinations in 60 seconds as possible. |
| **Stroop tests** | Attention and concentration were measured using the Stroop test, which comprises three trials. In the first trial, participants are asked to name the printed word of a card which contains color names in black. In the second trial, participants were asked to name the printed color of colored blocks. And last, in the third trial, participants were asked to name the color of the ink of the card that contains color names printed in different ink colors than the actual color name. |
| **Word Fluency Task (WFT)** | Verbal fluency was measured with the WFT. Participants were asked to provide as many animal names as possible during 60 seconds. |
| **15-Verbal word learning test (WLT)** | Immediate recall and delayed recall were measured with the WLT. First, participants were provided a list of 15 unrelated words and were asked to repeat them after five different trials and receiving another list of 15 unrelated words (WLTimm). This was asked again after 30 minutes (WLTdel). |
| **Purdue Pegboard test (PBB test)** | Unilateral and bilateral fine manual dexterity was quantified using the PPB. This test uses a pegboard comprising two rows, with 25 holes each and a number of pins. Participants get 30 seconds to place as many pins as possible in the holes in the prescribed order. First with the right hand, then the left and last using both hands. |

Supplementary table 2: Application of exclusion criteria per set.

| | Set 1 | Set 2 | Set 3 |
|---|---|---|---|
| **Eligible for inclusion** | **6.048** | **5.967** | **4.540** |
| Dementia at baseline | 79 | 45 | 27 |
| No dementia data | 4 | 10 | 7 |
| No follow-up | 24 | 22 | 17 |
| NAFLD exclusion criteria | | | |
| Viral hepatitis | 25 | 43 | 34 |
| Excessive alcohol* | 1.430 | 776 | 629 |
| Steatogenic drug use | 115 | 97 | 68 |
| Missing alcohol data | 396 | 397 | 315 |
| Invalid liver stiffness† | - | - | 143 |
| **Total participants excluded** | **2.073** | **1.390** | **1.240** |
| **Participants included** | **3.975** | **4.577** | **3.300** |

*Excessive alcohol: daily intake of >30 grams for male and > 20 grams for female. †According to the Boursier criteria.
Abbreviations: NAFLD, non-alcoholic fatty liver disease.

Supplementary table 3: baseline characteristics by NAFLD status for incident dementia analysis

| | Set 1: Fatty liver index | | | Set 2: Ultrasound | |
|---|---|---|---|---|---|
| | FLI < 30 n = 1335 | FLI 30 – 60 n = 1347 | FLI ≥ 60 n = 1293 | No NAFLD n = 2991 | NAFLD n = 1586 |
| **Demographics** | | | | | |
| Age (years) | 70.2 (8.3) | 70.3 (8.0) | 69.6 (7.7) | 69.9 (9.5) | 69.9 (8.5) |
| Female | 949 (71.1) | 745 (55.3) | 714 (55.2) | 1783 (59.6) | 926 (58.4) |
| Alcohol consumption | 1023 (76.6) | 1067 (79.2) | 978 (75.6) | 2523 (84.4) | 1343 (84.7) |
| Former/current smoking | 749 (56.5) | 873 (65.3) | 873 (67.7) | 1885 (62.1) | 1078 (68.1) |
| Educational level | | | | | |
| Low | 804 (60.7) | 780 (58.3) | 773 (60.4) | 1379 (46.6) | 858 (54.8) |
| Intermediate | 359 (27.1) | 400 (29.9) | 370 (28.9) | 911 (30.8) | 444 (28.4) |
| High | 162 (12.2) | 158 (11.8) | 136 (10.6) | 671 (22.7) | 263 (16.8) |
| **Physical examination** | | | | | |
| BMI ($kg/m^2$) | 23.5 (2.3) | 26.8 (2.3) | 30.9 (3.7) | 26.2 (3.7) | 30.2 (4.4) |
| Enlarged waist circumference* | 121 (9.1) | 601 (44.6) | 1077 (83.3) | 895 (29.9) | 1120 (70.6) |
| **Comorbidity** | | | | | |
| Metabolic syndrome | 230 (17.3) | 670 (49.9) | 1083 (84.0) | 841 (28.6) | 1028 (66.2) |
| Diabetes | 73 (5.5) | 157 (11.7) | 319 (24.7) | 313 (10.6) | 402 (25.8) |
| Stroke | 20 (1.5) | 22 (1.6) | 29 (2.2) | 82 (2.7) | 40 (2.5) |
| Hypertension | 759 (56.9) | 928 (69.0) | 1040 (80.7) | 2061 (68.9) | 1313 (82.8) |
| **Biochemistry / genetics** | | | | | |
| ALT (U/L) | 18 [15, 21] | 20 [16, 25] | 23 [18, 30] | 17 [14, 22] | 21 [16, 28] |
| GGT (U/L) | 17 [14, 23] | 23 [17, 30] | 31 [23, 44] | 21 [15, 30] | 28 [20, 39] |
| Cholesterol (mmol/L) | 5.72 (0.95) | 5.83 (0.98) | 5.80 (0.99) | 5.46 (1.10) | 5.35 (1.12) |
| Triglycerides (mmol/L) | 1.04 [0.84, 1.30] | 1.37 [1.08, 1.79] | 1.80 [1.39, 2.38] | 1.16 [0.91, 1.53] | 1.56 [1.18, 2.07] |
| APOE-ε4 | 374 (29.2) | 357 (27.4) | 331 (26.7) | 809 (29.0) | 328 (22.3) |

Data is presented as mean (SD), median [P25-P75] or n and percentage. Baseline characteristics are presented set 1 and set 2, stratified for NAFLD status. *Waist circumference > 102 cm for male and > 88 cm for female.

Abbreviations: APOE, apolipoprotein E; ALT, alanine transaminase; BMI, body mass index; FLI, fatty liver index; GGT, gamma glutamyl transpeptidase; NAFLD, non-alcoholic fatty liver disease.

Supplementary table 4: Mean difference of performance on cognitive tests between participants with NAFLD compared to no NAFLD and fibrosis compared to no fibrosis expressed in z-scores.

| Cognitive test | NAFLD | | | Fibrosis | | |
|---|---|---|---|---|---|---|
| | n | MD | 95% CI | n | MD | 95% CI |
| G-factor, z-score | 3574 | 0.032 | -0.029; 0.092 | 2657 | -0.172 | -0.307; -0.037* |
| LDST, z-score | 4414 | 0.042 | -0.019; 0.103 | 3197 | -0.201 | -0.335; -0.067* |
| Stroop test1*, z-score | 4425 | -0.047 | -0.114; 0.021 | 3204 | 0.199 | 0.053; 0.345* |
| Stroop test2*, z-score | 4424 | -0.079 | -0.148; -0.011* | 3203 | 0.135 | -0.015; 0.284 |
| Stroop test3*, z-score | 4415 | -0.046 | -0.110; 0.019 | 3199 | 0.179 | 0.041; 0.316* |
| WFT, z-score | 4503 | 0.000 | -0.065; 0.065 | 3248 | -0.132 | -0.274; 0.010 |
| WLTdel, z-score | 4193 | -0.027 | -0.093; 0.039 | 3028 | -0.024 | -0.165; 0.118 |
| WLTimm, z-score | 4193 | -0.011 | -0.076; 0.054 | 3027 | -0.006 | -0.145; 0.133 |
| WLTrecog, z-score | 4290 | -0.012 | -0.082; 0.058 | 3088 | -0.054 | -0.203; 0.096 |
| PPB test, z-score | 3982 | 0.051 | -0.009; 0.111 | 2958 | -0.086 | -0.219; 0.048 |

Higher scores indicate better cognitive function, except for Stroop tests. Results were obtained from linear regression analyses and Tukey all-pair comparisons method based on ANOVA models. Differences were calculated for the individual cognitive tests and G-factor for participants with NAFLD compared to those without NAFLD and for fibrosis compared to no fibrosis. Results were adjusted for age, sex, education level, smoking status, BMI, cholesterol, triglycerides, hypertension, stroke, diabetes, depression and APOE genotypes.

Abbreviations: *APOE*, apolipoprotein E; CI, confidence interval; G-factor, principle component scores of cognition tests; LDST, Letter-Digit Substitution test; MD, Mean difference; NAFLD, non-alcoholic liver disease; PPB test, Purdue Pegboard test; WFT, Word Fluency test; WLTdel, Word learning test, delayed recall; WLTimm, Word learning test, immediate recall; WLTrecog, Word learning test, recognition.

# Chapter 3.2 Sex-Stratified Associations Between Fatty Liver Disease and Parkinson's Disease: The Rotterdam Study

**Abstract**

Fatty liver disease was not associated with Parkinsonism or Parkinson's disease in an elderly European population, the Rotterdam Study, (n=8.848), neither in men nor women. Results were consistent either using non-alcoholic fatty liver disease (NAFLD) or metabolic-dysfunction associated fatty liver disease (MAFLD) as exposure defined by either fatty liver index (FLI) or ultrasound.

**Background**

Fatty liver disease is the most common chronic liver disease, with an estimated global prevalence of 33% in 2019.[168] While most patients with fatty liver disease do not encounter any symptoms, this liver disorder is associated with a range of hepatic and extra-hepatic complications.

Recently, fatty liver disease has been linked to Parkinson's disease (PD). However, this registry study from Korea with over 20.000 PD cases reported opposing results between men and women:[169] Women with fatty liver disease assessed by the fatty liver index (FLI) were at an increased risk (Hazard ratio (HR): 1.09; 95% Confidence interval (CI): 1.02-1.16) of PD, while men had a lower risk (HR: 0.86, 95%CI: 0.82-0.91).

Another registry study from Israel investigated whether fatty liver disease with active inflammation, known as steatohepatitis, was associated with PD. They reported that both men and women with steatohepatitis were more likely to have PD (Odds ratio (OR): 1.13, 95%CI: 1.08-1.19).[170] However, they did not report on the broader entity of fatty liver disease itself.

Considering the limited and conflicting evidence on the association between fatty liver disease and PD, we investigated this association within the ongoing Rotterdam Study.

**Participants**

For this specific study, we included participants who visited the study centre between 1997 and 2008 with data on liver disease. Participants were excluded in case of prevalent Parkinsonism or PD, incomplete data on the FLI components, no follow-up, or missing data on covariates. Detailed information on the Rotterdam Study isavailable elsewhere.[171]

**Exposure**

In accordance with validated criteria in Caucasian populations, the presence of fatty liver disease was defined as a FLI of ≥60 (range 0-100). FLI is an algorithm comprising BMI, waist circumference, gamma-glutamyl transferase (GGT), and triglycerides:

$$FLI = \frac{e^{0.953*\ln(triglycerides)+0.139*BMI+0.178*\ln(GGT)+0.053*waist\ circumference-15.745}}{1+e^{0.953*\ln(triglycerides)+0.139*BMI+0.178*\ln(GGT)+0.053*waist\ circumference-15.745}} * 100$$

A subset of the initial cohort underwent abdominal ultrasound (which has superior diagnostic accuracy) to assess fatty liver disease between 2009 and 2014. Subsequently, two largely overlapping entities for fatty liver diseases were used: (1) Metabolic dysfunction-associated fatty liver disease (MAFLD) was defined as the presence of fatty liver disease together with either overweight, diabetes, or two minor metabolic dysfunction criteria; (2) non-alcoholic fatty liver disease (NAFLD) was defined as the presence of fatty liver disease, in the absence of excessive alcohol consumption, viral hepatitis, or steatogenic drug use.

**Outcome**

Potential cases of Incident Parkinsonism and PD were identified by (1) data obtained during follow-up visits, (2) continuous monitoring of medical records, and/or (3) medication use. Potential cases were evaluated by a panel led by an experienced neurologist. Parkinsonism was defined as hypo- or bradykinesia with ≥ 1 cardinal sign (resting tremor, rigidity, or postural imbalance), or as a clinical diagnosis of Parkinsonism by a neurologist or geriatrician (if motor examination details were unavailable). Within those subjects, Parkinson's disease was defined with clinical history suggestive of Parkinson's disease verified by a neurologist or geriatrician, or with a positive response to dopaminergic treatment, or a dopamine transporter scan consistent with Parkinson's disease. The baseline was set on the date of liver assessment and follow-up ended at the diagnosis of Parkinsonism, PD, death, loss to follow-up, or 1st of January 2018.

**Statistics:** Cox regression models were used to quantify the risk of Parkinsonism and PD for the presence of MAFLD in the main analysis and NAFLD in the sensitivity analysis. Analyses were stratified for sex and multivariable models were adjusted for age, smoking status, and education levels as confounders based on literature. Finally, the analyses were repeated in the subset that underwent abdominal ultrasound.

**Results**

We included 9.364 participants between 1997 and 2008. Among them, 71 individuals were excluded with prevalent Parkinsonism or PD, 352 for lack of follow-up and 93 for missing data on covariates. In our final dataset (n = 8.848; age 64.7±9.6, 43.7% men) the mean FLI was 48.2±27.3 and 35.9% had FLI ≥ 60, which resulted in 35.8% MAFLD prevalence (45.5% among men, 28.3% among women). Additional baseline characteristics are available in **Table 2**.

In this study, 159 participants developed Parkinsonism and 74 PD during a median follow-up of 11 [IQR:9-17] years. MAFLD and NAFLD were neither associated with Parkinsonism nor PD in men nor women **(Table 2)**. Consistent results were obtained among 5.526 participants of our primary cohort that underwent abdominal ultrasound between 2009 and 2014.

**Context**

These results differ from the large Korean registry study with a median follow-up of 7 years, which reported fatty liver disease being a risk factor in women while in men, the presence of fatty liver disease reduced the risk of PD, as assessed by ICD-10 and registration codes.[169] The difference could be partly explained by FLI applicability in Asian people. To get comparable diagnostic accuracy in Asian populations as in non-Asians, the FLI cut-off for fatty liver disease may need to be lowered to ≥31 for men and ≥18 for women instead of ≥60 for both sexes.[172] Failing to use ethnic-specific cut-offs may distort any association in the study by Jeong *et al* and might explain

the low fatty liver disease prevalence of only 18.6% in men and 5.4% in women compared to the South Korea expected 41.1% in men and 20.3% in women.[173] In our study, 35.8% had fatty liver disease aligning with the expected prevalence in Europe, and results may thus be more representative of the entire caucasian fatty liver disease population.[168]

Furthermore, our cohort was older (mean age 65 years), therefore, it might not directly be compared to the study population investigated by Jeong and colleagues (mean age 54-55 years).[169] However, PD is uncommon in patients aged 40-59 (0.04-0.11%) while after that age, the prevalence increases rapidly to 1.9% in octogenarians.[174] Hence our study populations' age might be highly suitable to investigate associations between PD and NAFLD. Interestingly, when Jeong et al. focused on individuals aged ≥65 years (in which most PD cases occur) the sex differences in the association between fatty liver disease and PD disappeared.[169]

**Conclusion**

Fatty liver disease was not associated with Parkinsonism or PD in an elderly European population. Results were consistent either using NAFLD or MAFLD as exposure defined by either FLI or ultrasound.

Table 1: Characteristics at baseline and at abdominal ultrasound

| | **Baseline (1997-2008)** | **Abdominal ultrasound (2009-2014)** |
|---|---|---|
| | n = 8.848 | n = 5.526 |
| **Age** | 64.7 (9.6) | 69.4 (9.1) |
| **Men** | 3863 (43.7) | 2361 (42.7) |
| **Smoking** | 6142 (69.4) | 3729 (67.5) |
| **Education** | | |
| Low | 4632 (52.4) | 2671 (48.3) |
| Intermediate | 2601 (29.4) | 1666 (30.1) |
| High | 1615 (18.3) | 1189 (21.5) |
| **BMI** | 27.3 (4.2) | 27.6 (4.4) |
| **Metabolic syndrome** | 3846 (43.8) | 2697 (49.7) |
| **ALT** | 20 [15, 27] | 19 [15, 24] |
| **GGT** | 24 [17, 36] | 24 [17, 35] |
| **MAFLD** | 3169 (35.8) | 1902 (34.4) |
| **NAFLD*** | 2310 (34.1) | 1484 (34.5) |

*NAFLD prevalence was calculated in patients without secondary causes for steatosis and complete data on alcohol consumption (n = 6.773 [baseline] and n = 4.302 [abdominal ultrasound]).

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; GGT, gamma-glutamyltransferase; MAFLD, metabolic dysfunction associated fatty liver disease; NAFLD, non alcoholic fatty liver disease.

Table 2: Risk among men and women with fatty liver disease for Parkinsonism and Parkinson's Disease

| | Parkinsonism | | | Parkinson's Disease | | |
|---|---|---|---|---|---|---|
| | Cases / n | HR | 95% CI | Cases / n | HR | 95% CI |
| **Assessed by FLI** | | | | | | |
| MAFLD | | | | | | |
| Men | 89/3863 | 0.97 | 0.63 – 1.49 | 41/3863 | 1.04 | 0.56 – 1.94 |
| Women | 70/4985 | 0.91 | 0.54 – 1.55 | 33/4985 | 0.69 | 0.30 – 1.58 |
| NAFLD | | | | | | |
| Men | 62/2813 | 0.71 | 0.41 – 1.22 | 28/2813 | 0.81 | 0.37 – 1.78 |
| Women | 49/3960 | 1.09 | 0.59 – 2.02 | 25/3960 | 0.82 | 0.33 – 2.07 |
| **Assessed by ultrasound** | | | | | | |
| MAFLD | | | | | | |
| Men | 35/2361 | 1.35 | 0.68 – 2.69 | 14/2361 | 1.07 | 0.36 – 3.23 |
| Women | 23/3165 | 0.68 | 0.27 – 1.73 | 13/3165 | 0.62 | 0.17 – 2.27 |
| NAFLD | | | | | | |
| Men | 25/1754 | 1.01 | 0.43 – 2.35 | 9/1754 | 1.08 | 0.27 – 4.38 |
| Women | 21/2548 | 0.74 | 0.29 – 1.92 | 12/2548 | 0.66 | 0.18 – 2.45 |

Abbreviations: CI, confidence interval; FLI, fatty liver index; HR, hazard rate; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease. Results are given as HR and 95% CI for incident Parkinsonism or Parkinson's Disease as outcome. Analyses were adjusted for age, sex, smoking status and education.

# Chapter 4 Cardiac Biomarkers and Neurodegeneration

# Chapter 4.1 NT-proBNP and Changes In Cognition and Global Brain Structure: The Rotterdam Study

**Abstract**

**Objective:** To investigate the association between NT-proBNP and changes in cognition and global brain structure.

**Methods:** In the Rotterdam Study, baseline NT-proBNP was assessed at baseline from 1997 to 2008. Between 1997-2016, participants without dementia or stroke at baseline (n= 9,566) had repeated cognitive tests (every 3-6 years) for global cognitive function, executive cognitive function, fine manual dexterity, and memory. Magnetic resonance imaging of the brain was performed repeatedly at re-examination visits between 2005 and 2015 for 2,607 participants to obtain brain volumes, focal brain lesions, and white matter microstructural integrity as measures of brain structure.

**Results:** Among 9,566 participants (mean age 65.1±9.8 years), 5,444 (56.9%) were women, and repeated measures of cognition were performed during a median follow-up time of 5.5 years (range = 1.1-17.9), of whom 2,607 participants completed at least one brain imaging scans. Higher levels of NT-proBNP were associated with a faster decline of scores in the global cognitive function (P value = 0.003), and the Word-Fluency test (P value = 0.003), but were not related to a steeper deterioration in brain volumes, global fractional anisotropy and mean diffusivity, as indicators of white matter microstructural integrity, or focal brain lesions.

**Conclusions:** Higher baseline NT-proBNP levels were associated with a faster decline in cognition, however, no association with global brain structure was found.

**Keywords:** brain structure; cognition; MRI; NT-proBNP, change, repeated measurements

## Introduction

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is the inactive N-terminal fragment of proBNP, which is released by ventricular myocytes in response to increasing load in volume and pressure [175]. NT-proBNP is used in clinical settings as a well-established diagnostic marker of ventricular distention and cardiac dysfunction [176]. Recent evidence demonstrated that impaired cardiac function was associated with abnormal brain aging. The heart-brain-axis hypothesis proposes possible mechanisms linking cardiac dysfunction to brain health, including reduced cardiac output, atherosclerotic changes, and perturbated cerebral perfusion, which may result in cognitive decline and brain atrophy [177-180]. Especially given that normal cerebral blood flow is essential for brain function maintenance, hemodynamic dysfunction of the heart-brain axis may play a role in vascular brain injury and impairment of cognition [178]. This is supported by a substantial proportion of patients with heart failure also experiencing cognitive impairment [181]. As a promising non-invasive biomarker of this axis [182], NT-proBNP clinically indicates left ventricular dysfunction and also relates to a higher risk of dementia [183]. Evidence from cross-sectional studies also showed that elevated NT-proBNP levels were associated with markers of abnormal brain aging, including decreased brain issue volumes and worse cognitive function [24, 182, 184]. A more comprehensive insight into cerebral pathophysiology that links cardiac dysfunction to abnormal brain aging would benefit from clarifying the direction of the associations and assisting in unraveling the underlying mechanisms.

However, in previous studies, proposed associations between NT-proBNP and brain structure or cognition were based on single time-point measures of structural brain markers or cognitive tests at baseline, precluding any inferences on a temporal link between NT-proBNP and markers of brain aging or cognition. Also, although few studies reported an association between higher NT-proBNP levels and a faster decline in global cognitive function, using Mini-Mental State

Examination [185, 186], the application of limited cognitive tests in previous studies might impede an investigation of individual cognitive domains, such as memory and executive function. Analyses of a comprehensive test battery of cognition could unravel the effect of NT-proBNP on both global and specific functions of multiple cognitive domains, which could contribute to a better overview of the association between cardiac function and cognition.

In this study, we determined the longitudinal associations between NT-proBNP levels and changes in cognition and global brain structure with multiple measures in community-dwelling older adults.

**Methods**

The current study was embedded within the population-based Rotterdam Study, a prospective cohort study of which details have been described previously [45].

NT-proBNP was assessed during the study center visits at baseline in 9,946 participants between 1997 and 2008. These cohorts formed the baseline of this current study. Of these 9,946 participants, 380 persons with prevalent stroke (n=223) or dementia (n=157), were excluded. We used a standardized test battery for multiple cognitive domains to determine both longitudinal and cross-sectional associations between NT-proBNP levels and cognition. These cognitive tests were administered every 3 to 6 years at re-examination visits from 1997 to 2016. The cognitive test battery [93], including the Word-Fluency test (WFT), the Letter-Digit-Substitution task (LDST), the Stroop test, the Purdue Pegboard test (PPB test), and the 15-Word Learning test for delayed recall, immediate recall, and recognition (WLTdel, WLTimm, WLTrecog), were described in supplemental table 1. Total of 9,566 participants completed at least one of the following tests at baseline: the WFT (n=9,118), the LDST (n=9,039), the Stroop test 1 (n=8,862), the Stroop test 2 (n=8,846), the Stroop test 3 (n=8,821), the PPB test (n=3,137), the WLTdel (n=3,032), the WLTimm (n=3,033), and the WLTrecog (n=3,031). Among 8,667 participants at baseline, we also constructed a compound score for global cognitive function (G-factor) using only the WFT, the LDST, and the Stroop test 3 in principal component analysis, to prevent distortion by highly correlated tasks. The validity of g-factor has been tested within the Rotterdam Study [94]and accounted 64.1% of all variance in the cognitive tests, which is a typical proportion of variance that g-factor can explain [187].To guarantee the quality of the cognitive evaluation, cognitive tests of participants diagnosed with incident dementia (n=110) at a follow-up date of the cognitive test were excluded from their diagnosis onward.

Brain MRI scans were performed repeatedly at re-examination visits between 2005 and 2015 to obtain brain volumes, focal brain lesions, and white matter microstructural integrity as measures of brain structure. 2,775 participants had complete structural segmentation data of brain imaging at baseline. After the exclusion of 128 persons with prevalent stroke (n=64) or dementia (n=64) and 40 persons with cortical brain infarcts on MRI, 2,607 participants were included in the analyses. Numbers of cognitive tests or brain MRI scans during the follow-up were presented in supplemental table 2.

***Assessment of NT-pro-BNP***

Serum NT-proBNP levels were determined using electrochemiluminescence immunoassay at baseline (Elecsys proBNP, F Hoffman-La Roche Ltd, Basel, Switzerland) on an Elecsys 2010 analyzer, which measures concentrations ranging from 0.6 to 4130 pmol/L. Values below the detection limit are reported as < 0.6 pmol. Values above the measuring range are reported as > 4130 pmol/L or up to 8277 pmol/L for 2-fold diluted samples. The detailed information of NT-peoBNP measurement has been reported elsewhere [188]. NT-proBNP levels were measured in pmol/L.

***Brain structure***

Brain MRI scanning was performed on a single 1.5T MRI unit (General Electric Healthcare, Milwaukee, WI) with an 8-channel head coil. There were no software or hardware changes within the study period. The scans protocol included T1-weighted, proton density-weighted, fluid-attenuated inversion recovery and T2*-weighted gradient recalled echo sequences. Detailed information about brain MRI is presented in the supplementary methods. The distribution of brain volumes and white matter microstructure was transformed into a normal standardized distribution. Normalized scores (z-scores) for each scan were calculated by the individual raw score minus the

mean value of the whole population, divided by the population standard deviation. White matter hyperintensity volumes were log-transformed. Lower fractional anisotropy and higher mean diffusivity indicate worse white matter microstructural integrity [189].

***Cognition***

Similarly, z-scores were calculated for values of all cognitive tests. Lower scores on the WFT, the LDST, the PPB test, the WLTdel, the WLTimm, and the WLTrecog, and higher Stroop test scores indicate worse cognitive functions. As a compound score extracted from the principal component analysis, a higher G-factor indicates a better global cognitive function.

***Covariates***

We included potential covariates based on literature knowledge reporting an association with NT-proBNP, cognitive impairment, brain atrophy, or all, including age, sex, education levels (primary education, lower education, intermediate education, higher education), smoking status (never, former, current), body mass index (BMI, $kg/m^2$, calculated by weight [kg] divided by height [m] squared), systolic and diastolic blood pressure (mmHg), total and high-density lipoprotein cholesterol level (mmol/L), apolipoprotein E (*APOE*) genotype, depressive symptoms, and chronic comorbid conditions (diabetes mellitus type 2 and stroke) [37]. The majority of these variables are also related to cardiac function [190] and therefore were adjusted for in models. Blood samples were collected at the research center and used to determine cholesterol levels and DNA genotypes. *APOE* genotype was determined using a PCR or a bi-allelic TaqMan assay (rs7412 and rs429358) on labeled DNA samples. *APOE* genotype was classified into two groups: non-carriership or carriership of the *APOE*-ε4 allele, as *APOE*-ε4 has been recognized as major genetic risk factor for cognitive impairment, brain lesions and Alzheimer's disease onset [191]. Depressive symptoms were assessed with a validated version of the Centre for Epidemiologic Studies Depression (CES-

D) scale (range:0-60) [103]. Scores of 16 or greater were regarded as suggestive of clinically significant depressive symptoms [103]. Diabetes mellitus was defined as a fasting plasma glucose level ≥ 7 mmol/L, a non-fasting plasma glucose level ≥ 11.1 mmol/L, or the use of blood glucose-lowering medication [101]. Stroke was defined according to the World Health Organization criteria [102].

***Statistical analysis***

Data were expressed as mean ± standard deviation (SD) for normally distributed variables or as median (interquartile range [IQR]) for non-normally distributed variables among participants in tertiles of baseline NT-proBNP levels. Missing covariates were imputed with five-times imputation using a chained equation [52].

We used linear mixed-effect models to study associations between NT-proBNP and continuous outcomes and applied generalized estimating equations for dichotomous outcomes. NT-proBNP concentrations were firstly analyzed per one unit increase of log-transformed values to achieve normal distribution and next were categorized into tertiles with the lowest tertile as the reference. Similarly, values of white matter hyperintensity were naturally log-transformed due to non-normal distribution.

Based on linear curves of cognitive changes after the age of 65 years, as found in a previous study [192], the fixed-effect structure included NT-proBNP levels, age, quadratic age ($age^2$), time, quadratic follow-up time ($time^2$), sex, smoking status, education levels, body mass index, systolic and diastolic blood pressure, total and high-density lipoprotein cholesterol levels, prevalent diabetes mellitus, depressive symptom, and *APOE* genotype. The parameter of NT-proBNP ($\beta_{NT\text{-}proBNP}$) denoted an average effect of an increase in NT-proBNP on cognition, while other variables remain unchanged. For longitudinal analyses, we also added an interaction term between baseline age and

time, and an interaction term between NT-proBNP levels and time ($\beta_{NT\text{-}proBNP*time}$), which provides the average effect of NT-proBNP per unit increase on cognitive changes per year. In the random-effect structure, we used random intercepts and random linear slopes to incorporate individual response trajectories of cognition.

Regarding nonlinear trajectories of brain imaging markers with advancing age [193], the main difference from the above models of cognition was the different multiplicative terms in the fixed-effect structure. We included multiplicative terms between baseline age and time variables, including follow-up time and time$^2$, and interaction terms for the product of NT-proBNP levels and time variables (time and time$^2$), which together interpret the effect of NT-proBNP on the overall rate of changes in brain structure per year, along with the same random-effect structure as above. Analyses involving volumetric measures were additionally adjusted for intracranial volumes. Gray and white matter volumes were adjusted for each other. White matter microstructural measures (fractional anisotropy and mean diffusivity) were additionally adjusted for intracranial volumes and microstructural white matter measures (normal-appearing white matter and white matter hyperintensity). The associations between baseline NT-proBNP levels and the presence of microbleeds and lacunar infarcts were tested using the generalized estimating equation. We applied the same fixed-effect structure as the above linear mixed-effect model and used a first-order autoregressive correlation matrix.

In addition, we performed stratifications in sex, age (median), and *APOE*-ε4 allele carriership (carrier versus non-carrier). These were selected as possible effect modifiers based on previous literature and biological plausibility [193-195].

Because cut-off values of NT-proBNP are commonly used in clinical practice for the diagnosis of heart failure (900 pg/ml (=106.2 pmol/L) for likely heart failure, among populations aged > 50

years) [196], in sensitivity analyses, we repeated longitudinal analyses after excluding participants with NT-proBNP values > 106.2 pmol/L and/or any of following (cardio-) vascular diseases, including prevalent chronic kidney disease, coronary heart disease, and atrial fibrillation, and also excluding participants with missing information on heart disease at baseline, with regards to the confounding effect of these comorbidities on the associations [197].

Given that we included one determinant (NT-proBNP) and 18 outcomes (cognitive tests and brain MRI), which are partly interrelated, we ran permutation testing to ascertain the number of independent tests. For each outcome variable, 10,000 iterations of linear regressions using a random variable were performed. The minimum p value for each regression model (permutation) was extracted and these p values were sorted to define the significance threshold based on the 5% quantile (0.0038). We then calculated the number of independent tests by dividing 0.05 by this threshold, resulting in 13.1 independent tests. A multiple-testing adjusted p-value threshold (0.0039) was created by calculating the new significance threshold using the Sidák correction, $\alpha_n = 1 - (1 - \alpha)^{(1/n)}$, where n is the number of independent tests [198]. Data analyses were performed using R version 4.1.1 (Foundation for Statistical Computing, Vienna, Austria).

## Results

### *Characteristics of the study population*

As shown in Table 1, among 9,566 participants (mean age 65.1±9.8 years), 5,444 (56.9%) were women, and repeated measures of cognitive tests were performed during a median follow-up time of 5.5 years (range = 1.1-17.9), of whom 2,607 participants completed at least one brain imaging scans. (Supplemental table 3)

### *NT-proBNP and cognitive changes*

Participants with higher NT-proBNP levels had poorer performance on cognitive tests at baseline, including the G-factor, the LDST, the Stroop test, and the PPB test. (Figure 1A, Figure 1B, Figure 1C, Supplemental table 4)

Higher levels of NT-proBNP were linearly associated with a steeper decline in the G-factor, WFT scores, and PPB scores during the follow-up, but not with other cognitive tests (G-factor, interaction terms of time × NT-proBNP: mean difference of -0.003 per year per 1 unit increase in log-transformed NT-proBNP, 95% confidence interval (CI): (-0.005,-0.001), P value = 0.003; WFT, mean difference: -0.004 per year, 95%CI: (-0.006,-0.001), P value = 0.003; PPB test, mean difference: -0.006 per year, 95%CI: (-0.012,0.000), P value = 0.038). The association between NT-proBNP and PPB test was not statistically significant after multiple testing correction. Compared to participants in the lowest tertile of NT-proBNP, participants in the highest tertile showed a faster decline in the G-factor, the WFT, and the PPB test (G-factor, mean difference: -0.004 per year, 95%CI: (-0.009,0.000), P value = 0.046; WFT, mean difference: -0.007 per year, 95%CI: (-0.012,-0.002), P value = 0.011; PPB test, mean difference: -0.015 per year, 95%CI: (-0.028,-0.002), P value = 0.026). After multiple testing correction, these association turned out to be insignificant. (Figure 1A, Figure 1B, Figure 1C, Supplemental table 4)

***NT-proBNP and structural brain changes***

As presented in Figure 2A and Figure 2B, higher levels of NT-proBNP were associated with overall smaller volumes of total brain tissue, gray matter, and white matter, larger volumes of white matter hyperintensity, lower fractional anisotropy, and higher mean diffusivity at baseline. Baseline NT-proBNP was not associated with the steepness of changes in brain structure over time (interaction terms of time × NT-proBNP and time$^2$ × NT-proBNP). (Figure 2A, Figure 2B, Supplemental table 5)As presented in supplemental table 6, baseline NT-proBNP was not related to the occurrence of microbleeds and lacunar infarcts.

***Stratification***

Stratification by sex, age, or *APOE*-ε4 allele carriership on structural brain change did not show any differences between subgroups of the stratified factors. In cognitive analyses, we found a steeper deterioration in the G-factor, the WFT, and the Stroop test 3 with a per unit increase in NT-proBNP among males, but not in female participants. Participants aged above 60 years, and *APOE*-ε4 non-carriers, also showed a steeper decline in the G-factor, WFT, and PPB scores compared to younger participants or *APOE*-ε4 carriers. (Supplemental table 7.1-7.3)

NT-proBNP levels were not longitudinally associated with the rate of changes in the structural brain alteration and the occurrence of focal brain lesions in the stratification. (Supplemental table 8.1-8.3, Supplemental table 9.1-9.3)

***Sensitivity analysis***

After the exclusion of participants with any of (cardio-) vascular diseases and participants with missing information on heart disease at baseline, higher levels of NT-proBNP were still associated with a steeper decline for the WFT (P value = 0.007, not statistically significant after multiple testing correction), but not for the G-factor and PPB test. (data not shown)

## Discussion

In this study, we found that higher baseline NT-proBNP levels were associated with a steeper decline in cognitive function, in particular the G-factor, and WFT, but were not related to a faster deterioration of brain structure over time.

In line with previous studies (including one study from our group [183]), we confirmed a significant cross-sectional association between higher levels of NT-proBNP and cognitive impairment [183, 199, 200]. In analyses of cognitive changes, the main finding that higher NT-proBNP levels were also associated with a steeper decline in cognition was in accordance with a prior study [201]. However, the difference between the two studies lay in finding associations with different cognitive domains. In this study, higher levels of NT-proBNP were associated with a steeper decline in global cognitive function, and verbal fluency, but not with processing speed and memory, while a faster decline in processing speed, memory, and reaction was observed in the prior study. Part of the difference between the two studies could be explained by a difference in mean age between the study populations, as older age is an widely acknowledged risk factor of brain degeneration and affects cognitive performance via brain structural deterioration in specific regions. In the current study, our findings for change over time for the PPB disappeared after multiple testing adjustment, but the associations for the g-factor and WFT remained. This could indicate that NT-proBNP mechanisms are more involved in global cognitive function than in specific cognitive domains or specific brain structures. Given that the WFT may measure both executive functioning and verbal fluency, the WFT may capture a more global cognitive function than other individual cognitive tests. There are potential pathophysiological mechanisms behind NT-proBNP marking cognitive decline. First, this could be partly explained by cerebral hypoperfusion driven by reduced cardiac output [202, 203]. Cerebral hypoperfusion might influence cerebral blood flow, which could further

lead to dementia development [204, 205]. Moreover, cognitive improvement has been observed in patients after receiving cardiac transplantation, implying a close link between cardiac function and cognition [206]. Second, plasma natriuretic peptides have been involved in the regulation of blood-brain barrier integrity, synaptic transmission, and brain fluid homeostasis, and disruption in these functions has been suggested as a potential mechanism for cognitive decline [207, 208]. Third, structural brain alterations might play a role in mediating the association between NT-proBNP and cognitive function [184, 207]. As reported in the previous work of our group [24], a significant association was observed between increased NT-proBNP levels and subclinical brain damage. To explore potential pathways through which NT-proBNP affects cognitive changes, we also investigated the impact of NT-proBNP on structural brain changes over time.

The cross-sectional association between increased NT-proBNP levels and subclinical brain damage was also confirmed in this study, which was comparable to the previous finding reported by our group [24]. However, we did not observe any significant longitudinal association between the cardiac marker and structural brain changes, which contrasts with a prior study [209]. Sabayan et al, found that higher NT-proBNP was associated with a one percent annual decline in the volumes of total brain and gray matter [209]. These different findings might be explained by the older age of their study population. Older age was associated with a steeper decline in multiple brain imaging markers [193], which was also demonstrated by the significantly inverse interaction between age and follow-up time on structural brain changes in our models. However, our age stratification did not unravel the presence of the association between NT-proBNP and structural brain changes. More prospective studies are warranted to explore the cardiac function of structural brain alterations over time.

In stratification by age, a steeper decline in cognition was rather observed in older participants with higher NT-proBNP levels. Older age was related to reduced cerebral perfusion, which might further lead to cognitive damage [210]. Among the male but not female participants, we observed inverse associations between NT-proBNP and changes in cognition. Estrogen provides a beneficial effect on both neural cells and against cardiomyocyte apoptosis, by reducing inflammatory metabolic syndrome, acute-phase inflammatory processes, and oxidation, all modifying the effect of cardiac function on cognition [78, 211, 212]. However, evidence on a general population level is still lacking. More attention should focus on testing these hypotheses.

**Strengths and Limitations**

The major strength of this study was the large sample size of >9500 participants with cognitive tests and >2500 with brain MRI scans, and multiple repeated measures of cognition and brain structure over time. However, certain limitations need to be considered. One limitation was the weakness in determining the causality of associations concerning inherent restraints of observational studies, such as selection bias derived from missing data on repeated measurements due to inevitable dropout during the long period of follow-up. Second, our study only focused on general brain structure without regarding specific brain regions, which impedes establishing links between NT-proBNP, brain regions, and specific cognitive domains. In addition, magnetic field of 1.5T has its limitations, as a higher magnetic field increases the sensitivity of detecting abnormalities of the brain or changes in the brain over time, due to potentially better visualizing structural changes and characterizing signal properties of individual lesions, driven by gain in the signal-to-noise ratio (SNR) [50]. However, 1.5T MRI is a commonly used imaging method in research and in a clinical setting. The limitations of this imaging method regarding the brain structure measured used in this research is expected to be minimal, since we investigated the

association between NT-pro-BNP and global brain structures, rather than small brain regions; and are likely balanced by the advantage of having standardized image acquisition by keeping hardware and software stable over time. Third, since this study mainly focused on global measures of brain structure, including global FA and MD, regional effects may have been missed, which is a limitation. Last, this cohort was almost entirely European, with a median age above 65 years at baseline, this might restrict the extrapolation of our findings to multi-ethnic and younger populations.

**Conclusions**

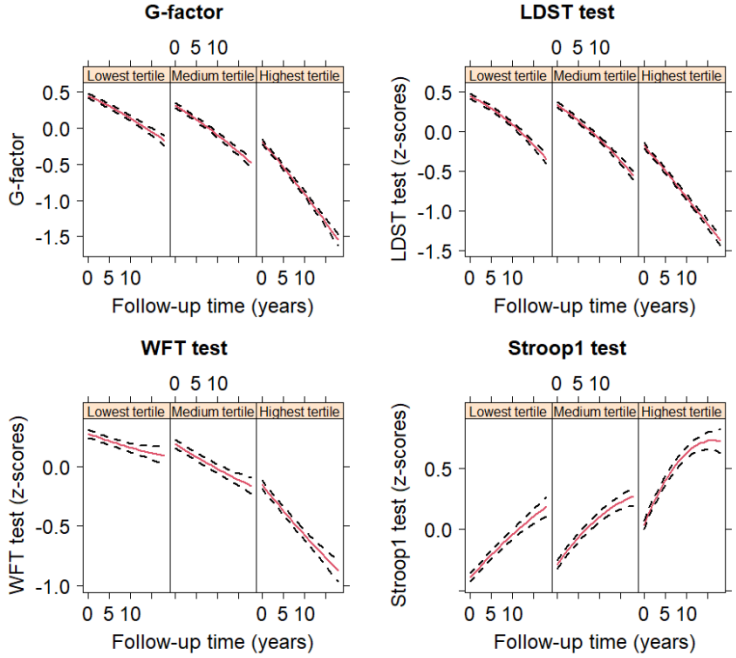
In conclusion, higher NT-proBNP levels were found to be associated with a faster decline in global cognition and the WFT, but not found with cognitive domains or global structural brain changes over time.

TABLE 1 Baseline characteristics of the population for cognition set

| Characteristics | Tertiles of NT-proBNP (pmol/L) | | | |
|---|---|---|---|---|
| | Total | Lowest tertile | Medium tertile | Highest tertile |
| | N=9566 | N=3191 | N=3187 | N=3188 |
| Age, years | 65.1 ± 9.8 | 59.9 ± 7.2 | 64.2 ± 8.6 | 71.0 ± 9.9 |
| Follow-up time, years | 5.5 ± 9.8 | 5.7 ± 9.5 | 5.6 ± 10.1 | 4.9 ± 1.6 |
| Female, (%) | 5444 (56.9) | 1364 (42.7) | 2060 (64.6) | 2020 (63.4) |
| NT-proBNP level, pmol/L | 18.7 ± 51.5 | 3.3 ± 1.3 | 8.4 ± 2.0 | 44.4 ± 83.4 |
| Diastolic blood pressure, mmHg | 78.8 ± 11.5 | 80.4 ± 10.4 | 78.6 ± 11.4 | 77.4 ± 12.4 |
| Systolic blood pressure, mmHg | 139.6 ± 21.1 | 135.0 ± 17.8 | 138.5 ± 20.4 | 145.3 ± 23.4 |
| Diabetes, (%) | 921 (9.6) | 296 (9.3) | 277 (8.7) | 348 (10.9) |
| Clinically relevant depressive symptoms, (%) | 786 (8.4) | 242 (7.7) | 265 (8.4) | 279 (9.0) |
| Cholesterol, mmol/L | 5.7 ± 1.0 | 5.8 ± 1.0 | 5.8 ± 1.0 | 5.7 ± 1.0 |
| High-density lipoprotein cholesterol, mmol/L | 1.4 ± 0.4 | 1.3 ± 0.4 | 1.4 ± 0.4 | 1.4 ± 0.4 |
| Body mass index, $kg/m^2$ | 27.3 ± 4.2 | 27.6 ± 4.1 | 27.1 ± 4.2 | 27.0 ± 4.2 |
| Educational level, (%) | | | | |
| Primary | 1166 (12.3) | 236 (7.5) | 380 (12.0) | 550 (17.4) |
| Lower | 3836 (40.5) | 1174 (37.1) | 1348 (42.6) | 1314 (41.7) |
| Intermediate | 2781 (29.3) | 978 (30.9) | 898 (28.4) | 905 (28.7) |
| Higher | 1699 (17.9) | 777 (24.5) | 538 (17.0) | 384 (12.2) |
| Smoking, (%) | | | | |
| Never | 3131 (33.0) | 996 (31.3) | 1050 (33.1) | 1085 (34.7) |
| Former | 4548 (48.0) | 1541 (48.5) | 1488 (46.9) | 1519 (48.6) |
| Current | 1797 (19.0) | 641 (20.2) | 632 (19.9) | 524 (16.8) |
| *APOE* genotype, (%) | | | | |
| ε4 allele carrier | 2584 (28.4) | 858 (28.2) | 879 (29.0) | 847 (27.9) |
| ε4 alleles non-carrier | 6520 (71.6) | 2188 (71.8) | 2147 (71.0) | 2185 (72.1) |

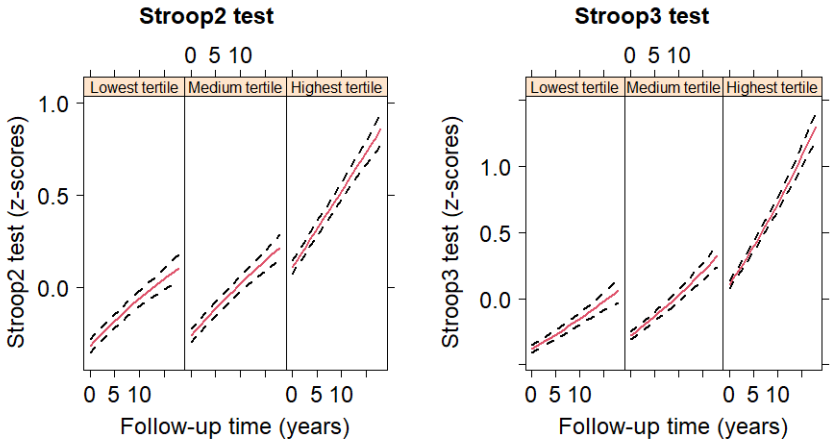
Definition of abbreviations: *APOE* = Apolipoprotein E; NT-proBNP = N-terminal pro-B-type natriuretic peptide. Data represent original data without imputed values. The missing proportion for different variables is listed as follows: age (0.1%), body mass index (1.2%), cholesterol (0.6%), high-density lipoprotein cholesterol (1.2%), education (0.9%), smoking (0.9%), diabetes (0.6%), diastolic blood pressure (0.5%), depressive symptom (1.9%), systolic blood pressure (0.5%) and *APOE* (4.8%).

FIGURE 1A Longitudinal associations between tertiles of baseline NT-proBNP levels and changes in individual cognitive tests (global cognitive function and executive cognitive function) over time (to be continued)



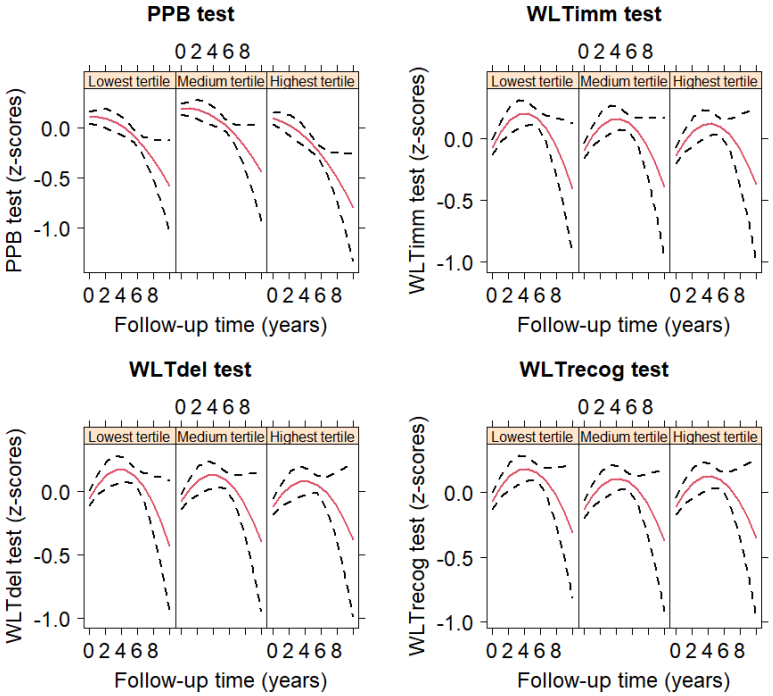

Definition of abbreviations: G-factor = principal component scores of global cognitive function; LDST = Letter-Digit Substitution test; WFT = Word Fluency test; NT-proBNP = N-terminal pro-B-type natriuretic peptide. MRI data are presented in tertiles and per 1-log increase in NT-proBNP level, using the linear mixed-effect model. All data in parentheses are 95% confidence intervals. Models were adjusted for age, age$^2$, time, time$^2$, sex, smoking status, education levels, body mass index, systolic and diastolic blood pressure, total and high-density lipoprotein cholesterol level, diabetes mellitus, depressive symptom, *APOE* genotype, and an interaction term of the product of follow-up time and baseline age. Red solid lines represent the marginal (group) changes in individual cognitive tests, and black dashed lines represent the 95% confidence intervals based on fully adjusted models. Lower scores on the G-factor, the WFT, the LDST, and higher Stroop test scores indicate worse cognitive functions.

FIGURE 1B Longitudinal associations between tertiles of baseline NT-proBNP levels and changes in individual cognitive tests (executive cognitive function) over time



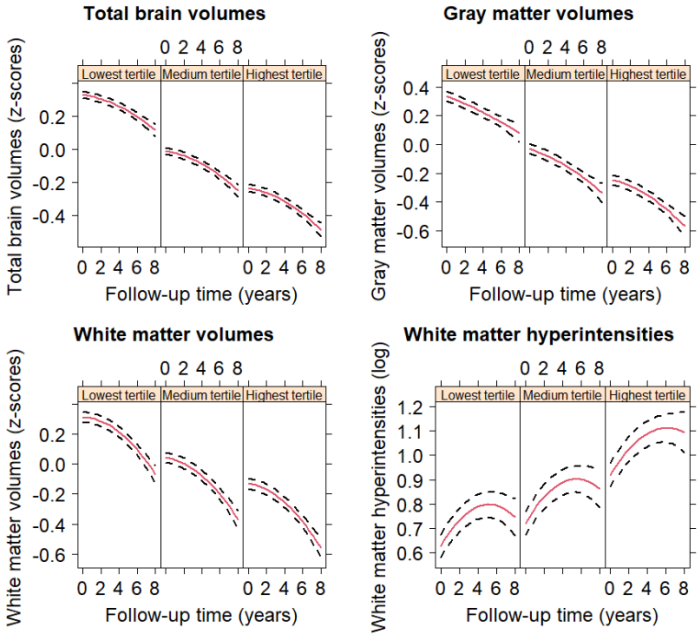

Definition of abbreviations: NT-proBNP = N-terminal pro-B-type natriuretic peptide. MRI data are presented in tertiles and per 1-log increase in NT-proBNP level, using the linear mixed-effect model. All data in parentheses are 95% confidence intervals. Models were adjusted for age, age$^2$, time, time$^2$, sex, smoking status, education levels, body mass index, systolic and diastolic blood pressure, total and high-density lipoprotein cholesterol level, diabetes mellitus, depressive symptom, *APOE* genotype, and an interaction term of the product of follow-up time and baseline age. Red solid lines represent the marginal (group) changes in individual cognitive tests, and black dashed lines represent the 95% confidence intervals based on fully-adjusted models. Higher Stroop test scores indicate worse cognitive functions.

FIGURE 1C Longitudinal associations between tertiles of baseline NT-proBNP levels and changes in individual cognitive tests (fine manual dexterity and memory) over time



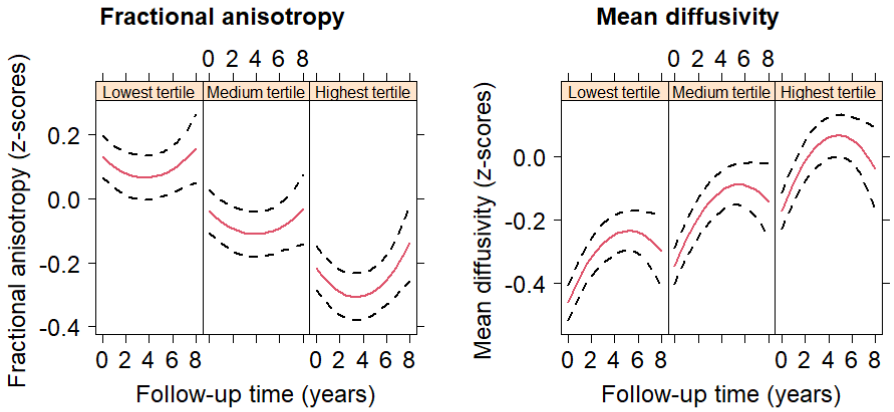

Definition of abbreviations: PPB test = Purdue Pegboard test; WLTdel = Word learning test, delayed recall; WLTimm = Word learning test, immediate recall; WLTrecog = Word learning test, recognition; NT-proBNP = N-terminal pro-B-type natriuretic peptide. MRI data are presented in tertiles and per 1-log increase in NT-proBNP level, using the linear mixed-effect model. All data in parentheses are 95% confidence intervals. Models were adjusted for age, age$^2$, time, time$^2$, sex, smoking status, education levels, body mass index, systolic and diastolic blood pressure, total and high-density lipoprotein cholesterol level, diabetes mellitus, depressive symptom, *APOE* genotype, and an interaction term of the product of follow-up time and baseline age. Red solid lines represent the marginal (group) changes in individual cognitive tests, and black dashed lines represent the 95% confidence intervals based on fully adjusted models. Lower scores on the PPB test, the WLTdel, the WLTimm, and the WLTrecog indicate worse cognitive functions.

**FIGURE 2A** Longitudinal associations between tertiles of baseline NT-proBNP levels and changes in brain volumes and white matter microstructure




Definition of abbreviations: NT-proBNP = N-terminal pro-B-type natriuretic peptide MRI data are presented in tertiles of NT-proBNP level, using the linear mixed-effect model. All data in parentheses are 95% confidence intervals. Models were adjusted for age, age$^2$, time, time$^2$, sex, smoking status, education levels, body mass index, systolic and diastolic blood pressure, total and high-density lipoprotein cholesterol level, diabetes mellitus, *APOE* genotypes, and interaction terms of the product of follow-up time or time$^2$ and baseline age. Analyses involving volumetric measures were additionally adjusted for intracranial volumes. Gray and white matter volumes were adjusted for each other. Microstructural measures were additionally adjusted for phase encoding direction, intracranial volumes, and microstructural white matter measures (volumes of the normal-appearing white matter and white matter hyperintensity). Red solid lines represent the marginal (group) changes in brain structure, and black dashed lines represent the 95% confidence intervals based on fully-adjusted models.

FIGURE 2B Longitudinal associations between tertiles of baseline NT-proBNP levels and changes in white matter microstructure integrity




Definition of abbreviations: NT-proBNP = N-terminal pro-B-type natriuretic peptide MRI data are presented in tertiles of NT-proBNP level, using the linear mixed-effect model. All data in parentheses are 95% confidence intervals. Models were adjusted for age, age$^2$, time, time$^2$, sex, smoking status, education levels, body mass index, systolic and diastolic blood pressure, total and high-density lipoprotein cholesterol level, diabetes mellitus, *APOE* genotypes, and interaction terms of the product of follow-up time or time$^2$ and baseline age. Microstructural measures were additionally adjusted for phase encoding direction, intracranial volumes, and microstructural white matter measures (volumes of the normal-appearing white matter and white matter hyperintensity). Red solid lines represent the marginal (group) changes in brain structure, and black dashed lines represent the 95% confidence intervals based on fully-adjusted models. Lower fractional anisotropy and higher mean diffusivity indicate worse white matter microstructural integrity.

SUPPLEMENTARY MATERIAL

Supplementary materials are available on:

# Chapter 4.2 Cardiac Biomarkers and the Risk of Parkinsonism and Parkinson's Disease: The Rotterdam Study

# Chapter 5 Bone Mineral Density and Neurodegeneration

# Chapter 5.1 Association of Bone Mineral Density and Dementia: The Rotterdam Study

**Abstract**

**Background & Objective:** Low bone mineral density and dementia commonly co-occur in the elderly, with bone loss accelerating in dementia patients due to physical inactivity and poor nutrition. However, uncertainty persists over the extent to which bone loss already exists prior to the onset of dementia. Therefore, we investigated how dementia risk was affected by bone mineral density at various skeletal regions in community-dwelling older adults.

**Methods:** In a prospective population-based cohort study, bone mineral density at the femoral neck, lumbar spine, and total body and the trabecular bone score were obtained using dual-energy X-ray absorptiometry (DXA) in 3,651 participants free from dementia between 2002-2005. Persons at risk of dementia were followed up until 1 January 2020. For analyses of the association between bone mineral density at baseline and the risk of incident dementia, we used Cox proportional-hazards regression analyses, adjusting for age, sex, educational attainment, physical activity, smoking status, body mass index, systolic blood pressure, diastolic blood pressure, cholesterol level, high-density lipoprotein cholesterol, history of comorbidities (stroke and diabetes mellitus), and *APOE* genotype.

**Results:** Among the 3,651 participants (median age 72.3±10.0 years, 57.9% women), 688 (18.8%) developed incident dementia during a median of 11.1 years, of whom 528 (76.7%) developed Alzheimer's disease. During the whole follow-up, participants with lower bone mineral density at the femoral neck (per SD decrease) were more likely to develop all-cause dementia (Hazard ratio [HR] $_{\text{total follow-up}}$: 1.12, 95% Confidential interval [CI]: 1.02-1.23) and Alzheimer's disease (HR $_{\text{total follow-up}}$: 1.14, 95% CI: 1.02-1.28). Within the first ten years following baseline, the risk of dementia was greatest for groups with the lowest tertile of bone mineral density (femoral neck bone mineral

density, $HR_{0\text{-}10years}$ 2.03; 95% CI, 1.39–2.96; total body bone mineral density, $HR_{0\text{-}10years}$ 1.42; 95% CI, 1.01–2.02; trabecular bone score, $HR_{0\text{-}10years}$ 1.59; 95% CI, 1.11–2.28).

**Conclusions:** In conclusion, participants with low femoral neck and total body bone mineral density and low trabecular bone score were more likely to develop dementia. Further studies should focus on the predictive ability of bone mineral density for dementia.

**Keywords:** Bone mineral density, femoral neck, lumbar spine, trabecular bone score, dementia, Alzheimer's disease.

## Introduction

Over 45 million people worldwide suffer from dementia, and this figure is estimated to double in the next two decades [82, 253]. The initial step in mapping the health journey of persons developing dementia and understanding how systemic changes contribute to the pathogenesis and clinical manifestation of dementia is crucial to the development of efficacious preventive strategies. Numerous chronic conditions, including cardiac disorders, diabetes, lung function impairment, and kidney disease [254, 255], have been related to dementia. Several studies have also suggested a link between bone mineral density and dementia or cognitive impairment [256, 257], most likely explained by shared risk factors, such as old age, subclinical hyperthyroidism [258], sarcopenia [259], sex steroids [260], physical inactivity [259], and vitamin D deficiency [261]. While it remains unclear whether bone health itself may be causally linked to dementia, it is an important predictor of fracture [262, 263], which is an important source of morbidity in dementia and can lead to loss of independence [264]. Therefore, temporally linking bone mineral density to dementia can provide important insights into how comorbidities occur at the prodromal phase of dementia. This in turn can aid in preventive strategies aimed at optimizing the health and care of dementia patients, including maintaining functional independence.

Previous studies focused solely on bone mineral density, assessed through DXA scanning of clinically relevant skeletal sites i.e., femoral neck and lumbar spine [265]. More recently, trabecular bone score has been developed, which is a novel gray-level texture measurement connected to bone microarchitecture and other structural features[266, 267]. The trabecular bone score offers further details, such as bone microarchitecture, that are not possible to infer from the areal bone mineral density.

In this study, we aimed to investigate the association between bone mineral density, measured across multiple skeletal sites, and dementia risk in community-dwelling older adults.

## Methods

### Study population

This study was performed within the Rotterdam Study, a prospective ongoing cohort study that started in 1990, and all participants aged ≥ 45 years were invited for studying chronic diseases in the general population [45]. The Rotterdam cohort comprises one original cohort (RS-I, initiated in 1990, 7,983 participants aged ≥55 years) and other two cohorts (RS-II, starting from 2000, 3,011 participants aged ≥ 55 years; and RS-III, starting from 2006, 3,932 aged ≥ 45 years), respectively. Every four to five years, participants participated in consecutive follow-up home interviews and diverse physical tests at the medical research center. The study has been approved by the medical ethics committee of the Erasmus Medical Centre (Rotterdam, the Netherlands), and the review board of the Netherlands Ministry of Health, Welfare and Sports (1068889-159521-PG). At baseline between 2002 and 2005, participants of RS-I and RS-II underwent a bone scans. A total of 3,651 persons with DXA scans and without prevalent dementia were finally included in this study (Figure1).

### Measurements of bone mineral density

Bone mineral density at the femoral neck, the lumbar spine, and the total body were measured using specific Prodigy DXA densitometer as described elsewhere [268]. A trained technician performed and verified all bone scans and made adjustments when necessary. A total of 3,651 had completed at least one scan of bone mineral density, of whom 3,584 participants had data on the bone mineral density at the femoral neck, 3,608 at the lumbar spine, and 3,633 of the total body.

### Measurement of trabecular bone score

Trabecular bone score was calculated using the trabecular bone score iNsight software (Med-Imaps, Geneva, Switzerland) [266]. Briefly, the trabecular bone score is a novel gray-level texture measurement [266, 267], and a higher score indicates stronger and more fracture-resistant

microarchitecture [269]. For each region of measurement (the $L_2$, $L_3$, and $L_4$ vertebrae) [269], trabecular bone score was assessed using gray-level analysis of the DXA images and the methodology of the trabecular bone score has been described elsewhere [267]. Trabecular bone score was available for 3,573 participants at baseline.

**Dementia assessment**

The Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) were used for detecting dementia at baseline and subsequent visits [45]. Further investigation and interview, including the Cambridge Examination for Mental Disorders of the Elderly, were conducted on participants with a MMSE score < 26 or GRS score > 0. Additionally, the study database was electronically linked to medical records from general practitioners and the regional institute for outpatient mental health care, allowing for the ongoing monitoring of incident dementia. When necessary, cognition tests and clinical neuroimaging were utilized to confirm dementia subtypes [45]. An adjudication panel headed by a consultant neurologist established the final diagnosis in accordance with the accepted dementia diagnostic criteria (Diagnostic and Statistical Manual of Mental Disorder, Third Edition-Revised: DSM-III-R) and Alzheimer's disease (AD) (National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association: NINCDS–ADRDA). The follow-up were stopped until meeting any of following scenarios, including incident dementia diagnosis, death, loss to follow-up, or 1 January 2020, whichever came first.

**Covariables**

Potential covariables were selected according to literature evidence demonstrating an association with bone mineral density, dementia, or both [37, 58, 268]. Baseline covariables included age, sex, education level (primary education, lower education, intermediate education, higher education), smoking status (never, former, current), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), total cholesterol level (mmol/L), high-density lipoprotein cholesterol

(mmol/L), triglycerides (mmol/L), body mass index (kg/m$^2$, calculated by weight [kg] divided by height [m] squared), measurements of physical activity and chronic disorders (diabetes and stroke). For determining apolipoprotein E (*APOE*) genotype, a PCR was used in RS-I and a bi-allelic TaqMan assay (rs7412 and rs429358) was employed on labelled DNA samples in both RS-II and RS-III. This study included the first two sub-cohorts (RS-I and RS-II). *APOE*-ε4 allele represented carrier ship of at least one ε4 alleles. Participants were divided into three different groups: high genetic risk (ε2ε4, ε3ε4, or ε4ε4), intermediate risk (ε3ε3) or low risk (ε2ε2 or ε2ε3) for dementia [270].

**Statistical analysis**

For baseline characteristics, normally distributed variables are described as mean ± standard deviation (SD) and non-normally distributed continuous variables as median (interquartile range) among women and men.

Cox proportional-hazards models were used for investigating the association between bone mineral density and dementia risk. Follow-up time started on the baseline date of bone scan and ended until the date of diagnosis of dementia, death, loss to follow-up, or 1 January 2020. Schoenfeld residuals were calculated for checking the proportional hazards assumption. And the proportional hazards assumptions were not violated, if P-values were above 0.05. We used Kaplan–Meier survival curves to map group differences in bone mineral density and trabecular bone score tertiles with respect to time to dementia. Cox proportional hazard models were adjusted for age, sex, *APOE* genotypes, education attainment, physical activity, smoking status, body mass index, systolic blood pressure, diastolic blood pressure, cholesterol level, high-density lipoprotein cholesterol, and history of chronic disorders (stroke and diabetes mellitus). Age and sex are two strong risk factors for both low bone mineral density, as bone mineral loss is manifested after age 50 years or menopause [271, 272], therefore, the tertile categories of bone mineral density were derived by generating tertiles from the residuals of linear regression

models adjusted for age (continuously) and sex. Effects of bone mineral density were also assessed when expressed as per one standard deviation (SD) decrease. Associations were determined by measuring the effects of each tertile or per one SD decrease of bone mineral density at the femoral neck, the lumbar spine, and total body, and trabecular bone score on the dementia risk.

As a consequence of the proportionality assumption of the Cox model being violated in some analyses, stratified Cox models by incremental epochs of follow-up time were used to examine how the aforementioned risk of incident dementia changed over follow-up duration (extending epochs e.g., baseline to 5 years, baseline to 10 years, baseline to over 10 years). In addition, we stratified analyses by sex and *APOE*-ε4 allele carrier ship (carrier versus non-carrier), which were suggested as possible effect modifiers [58, 273, 274].

A p-value of <0.05 was considered statistically significant. Data analyses were done using R version 3.6.0 (www.R-project.org.) (http://CRAN.R-project.org/package=lme4). Missing covariates were computed using predictive mean matching for numeric variables and logistic regression for binary variables using the MICE package [52].

## Results

### Clinical characteristics

As shown in Table1, among 3,651 participants (median age 72.3±10.0 years), 2113 (57.9%) are women. During a median follow-up duration of 11.1 years, 688 (18.8%) developed incident dementia, of whom 528 (76.7%) developed Alzheimer's disease.

### Bone mineral density and dementia risk over incremental epochs of follow-up duration

Throughout the whole follow-up, lower bone mineral density at the femoral neck (per SD decrease), not at other bone sites, was related to a higher risk of all-cause dementia (Hazard ratio $[HR]_{\text{total follow-up}}$: 1.12, 95% Confidential interval [CI]: 1.02-1.23) and Alzheimer's disease ($HR_{\text{total follow-up}}$: 1.14, 95% CI: 1.02-1.28) (Table 2). As presented in eTable 1, results were similar when we categorized individuals by bone mineral density tertiles: the highest risks were observed for dementia and Alzheimer's disease in the lowest group.

Within the first ten years following baseline, associations were greatest between lower bone mineral density (per SD decrease) and a higher risk of all-cause dementia (femoral neck bone mineral density, $HR_{0\text{-}10\text{years}}$ 1.43; 95% CI, 1.19–1.72; total body bone mineral density, $HR_{0\text{-}10\text{years}}$ 1.22; 95% CI, 1.00–1.47), and Alzheimer's disease (femoral neck bone mineral density, $HR_{0\text{-}10\text{years}}$ 1.52; 95% CI, 1.20–1.92). The hazard ratios for incident all-cause dementia comparing the lowest tertile of the femoral neck bone mineral density, total body bone mineral density, and trabecular bone score with the highest tertile were 2.03 (95% CI, 1.39–2.96), 1.42 (95% CI, 1.01–2.02), and 1.59 (95% CI, 1.11–2.28) separately (Table 2). Similar results remained only between femoral neck bone mineral density, trabecular bone score, and the risk of Alzheimer's disease, which were listed in eTable 1.

As shown in Table 2 and eTable 1, only bone mineral density at the femoral neck was related to all-cause dementia occurrence over the first five years of the follow-up ($HR_{0\text{-}5years}$ 2.13; 95% CI, 1.28–3.57, per SD decrease).

**Kaplan-Meier curves of dementia-free survival by levels of bone mineral density**

As presented in Figure 2 and eFigure 1, within the first 5 years during the follow-up, the curves of dementia-free or Alzheimer's disease-free probability were nearly overlapped at all tertile levels of the bone mineral density, but the curve at the lowest tertile of the femoral neck bone mineral density started to fall faster than that at the highest tertile later on. Similar temporal curve trends for dementia-free probability were also observed for the total body bone mineral density and trabecular bone score, but not for the lumbar spine bone mineral density.

**Stratification**

When stratified by sex and *APOE*-ε4 carriership, significant associations were found between lower femoral neck bone mineral density (the lowest tertile vs the highest tertile) and a higher risk of all-cause dementia in men (HR 1.56; 95% CI, 1.12–2.16), but not in women (HR 1.13; 95% CI, 0.87–1.47); and non-*APOE*-ε4 carriers (HR 1.36; 95% CI, 1.04–1.76), but not in *APOE*-ε4 carriers (HR 1.16; 95% CI, 0.84–1.60). Significant inversed associations were also presented between low trabecular bone score and dementia risk (Figure 3). Stratification for the HR estimates of Alzheimer's disease was represented in eFigure 2. Statistically significant interactions were observed between sex and low trabecular bone scores (P=0.02), and between *APOE*-ε4 carriership and low trabecular bone scores (P=0.01) (data not presented).

**Discussion**

In this study, low femoral neck and total body bone mineral density and low trabecular bone score were associated with an increased risk of dementia. The associations were strongest in the first ten years of follow-up.

Participants with low bone mineral density at the femoral neck had an increased risk of dementia in both the current study and previous prospective studies [275, 276]. It has also been demonstrated that participants with low femoral neck bone mineral density may also experience structural brain changes, including declined white matter volume, increased white matter hyperintensity volume, occurrence of silent brain infarction, and progression of parenchymal atrophy [277, 278]. A small cross-sectional study found that low total body bone mineral density was common in the earliest clinical stages of Alzheimer's disease and was related to brain atrophy and memory decline [34], which was supported by the significant association between total body bone mineral density and dementia risk in this study. Potential pathophysiological mechanisms behind low bone mineral density being a prodrome of dementia might include the effect of amyloid-beta on suppressing osteoblast proliferation and enhancing osteoclast activity [279, 280], and/or impact of systemic Wnt/Beta-catenin signaling deficits on impeding osteoblast differentiation and bone formation [281, 282]. Apart from the above pathway, bone-derived proteins, such as osteopontin, osteocalcin, sclerostin might also impact both bone loss and dementia progression [283]. Moreover, the loss of cognition preceding dementia inevitably influences quality of life among elderly by modifying nutrition intake and self-care ability, which further accelerates the loss of bone mineral density and increases fracture risk with aging [284, 285].

Concerning scarcity of evidence on the association between bone microarchitecture and dementia, an inverse association was observed between trabecular bone score and dementia risk. Low

trabecular bone score was associated with a weak and less fracture-resistant microarchitecture [269], and consequently also with fractures [262]. As the disease progresses, participants with subclinical dementia could experience changes in body composition [286] and confront with an increased risk of fracture [287], which was reported as an independent risk of dementia [288]. This suggests that low trabecular bone score might occur as a prodromal feature of dementia. Further evidence from prospective studies is warranted to demonstrate causality of the association.

Our findings did not support a link between lumbar spine bone density and dementia risk, which contrasts with findings of prior studies [289, 290]. Low bone mineral density at the lumbar spine was associated with cognitive decline over a 3-years follow-up period in a Korean middle-aged community-dwelling population [289]. Moreover, a Chinese cohort study (n=946) reported an association between low lumbar spine bone mineral density and increased risks of Alzheimer's disease and the conversion from mild cognitive impairment to the onset of dementia [290]. Different findings might result from relatively small sample size, short follow-up time, or cross-sectional design of previous studies.

Our study shows that femoral neck BMD is the most robustly associated with incident dementia. There are biological differences between skeletal sites which may explain these differences in effect. Bones within the lumbar spine consist predominantly of trabecular bone with a thin sheet of cortical bone surrounding them. In contrast long bones, like those of the femur, are comprised predominantly of a thicker sheet of cortical bone and a thin inner layer of trabecular bone. Cortical and trabecular bone differ in their material, mechanical and functional properties. Thus, changes to cortical BMD could affect dementia risk more strongly than trabecular BMD and this could be reflected in the differences in associations between sites. Furthermore, BMD of the femoral neck and total hip has been shown to decrease more rapidly with age in comparison to other skeletal

sites[291, 292]. Risk factors, such as poor diet and physical activity, may impact these bones differentially, in terms of their composition and rate of decline, which in turn may explain the differential associations with dementia. However, the exact mechanism remains unclear and should be the focus of future study.

Our study added extra knowledge to previous findings that associations change with time, with the strength of the effect decreasing with increasing follow-up time. This suggests that total bone mineral density and trabecular bone score might occur as prodromal features instead of causes of dementia and related toxic protein accumulation in the brain. In other words, persons with subclinical, incipient dementia may have poor bone health due to the dementia process instead of vice versa. Alternatively, participants with a low level of bone mineral density are at a high risk of falls and other mortalities, especially with longer follow-up duration, and thus death as a competing risk may also affect the associations. Additionally, the results in the first five years of follow-up would be unstable. The small number would primarily affect the power of these analyses, reflected in wider confidence intervals. The effect size itself would not necessarily be affected. Nevertheless, given the limited number of dementia cases, the interpretation of this part result should be taken with caution.

In contrast to the finding of a prior study [275], our study suggested that low bone mineral density increased the risk of dementia in males, but not in females. Tan ZS, et al [275] reported an increased risk of Alzheimer's disease only among women with low bone mineral density at the femoral neck, which indicates potentially protective effect of estrogen on mediating the negative association through inhibiting bone resorption and deterring neuronal apoptosis, atherosclerosis and oxidative stress [293, 294]. Although the risk of Alzheimer's disease decreased after taking estrogen replacement [295], this was contradicted by another study [296]. In addition, little evidence supports sex differences

in the associations of low bone mineral density with brain atrophy [277]. Future research is therefore needed to explore these hypotheses further.

The aim of our study was not necessarily etiologic, but instead to demonstrate the pattern of association. Indeed, we do not feel that BMD per se is causally related to dementia. Unraveling such etiologic link could for instance be a topic of study in Mendelian Randomization studies. Nevertheless, as an indicator of dementia risk, intervening in BMD may improve clinical care of these persons, especially considering the multi-comorbidities and polypharmacy that are highly preventive in this group.

The major important strength of our study is the relatively long follow-up time (mean 11.1±2.9 years) and sufficient incident cases of dementia (n=688). One limitation of this study lies in the weakness in determining the causality of associations concerning inherent restraints of observational study, including unmeasured confounders such as vitamin D and K and osteoporosis medications, although a large number of covariables were adjusted for in models. Future studies are warranted to assess the effect of these factors on the association. Additionally, another weakness of this study is the violation of the proportionality assumption in some cox models. However, we performed stratification by incremental epochs of follow-up duration extending from the baseline. Finally, due to the fact that our participants were primarily of European origin, with a mean age over 70 years at baseline, this might restrict the extrapolation of our findings to other populations/ethnicities and younger populations.

**Conclusion**

In conclusion, participants with low femoral neck and total body bone mineral density and low trabecular bone score were more likely to develop dementia. Further studies should focus on the predictive ability of bone mineral density for dementia.

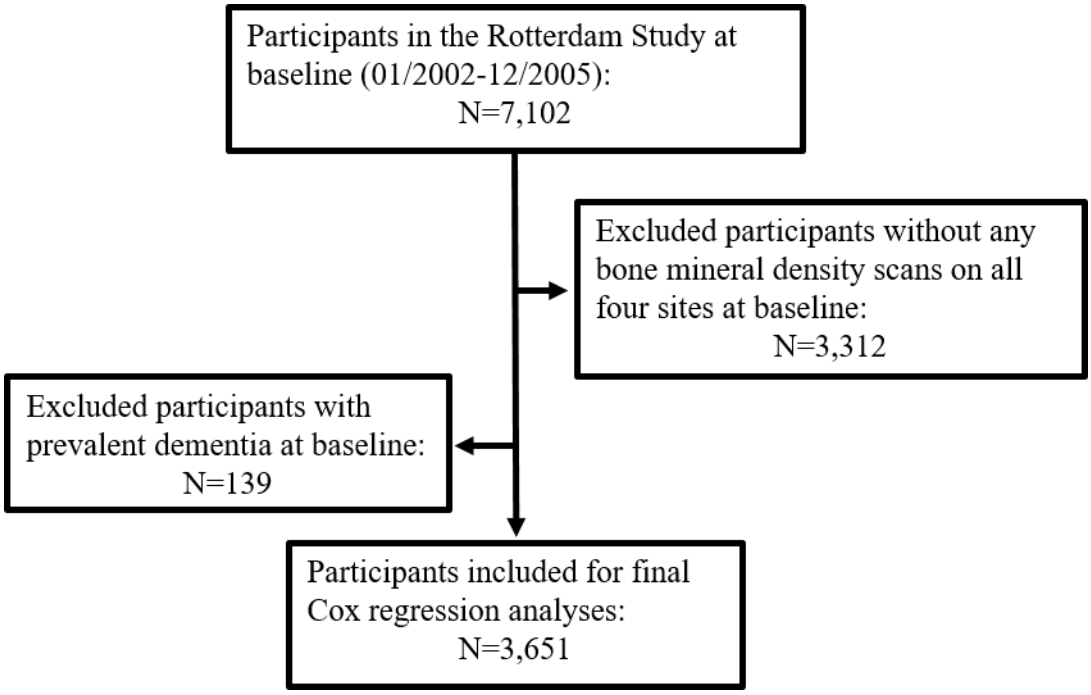



FIGURE 1 Flow chart for participants with bone mineral density scans included in the study

TABLE 1 Baseline characteristics of participants in longitudinal analyses

| | Men | Women | Total |
|---|---|---|---|
| N, (%) | 1538 (42.1) | 2113 (57.9) | 3651 (100) |
| Follow-up, years | 10.9 (4.3) | 11.2 (2.2) | 11.1 (2.9) |
| Age, years | 72.3 (9.5) | 72.3 (10.4) | 72.3 (10.0) |
| Body mass index, Kg/m$^2$, (%) | | | |
| Normal 18.5-24.9 | 389 (25.3) | 588 (27.8) | 977 (26.8) |
| Underweight < 18.5 | 4 (0.3) | 19 (0.9) | 23 (0.6) |
| Overweight 25-30 | 863 (56.1) | 948 (44.9) | 1811 (49.6) |
| Obesity > 30 | 282 (18.3) | 558 (26.4) | 840 (23.0) |
| Alcohol, g/day | 12.1 (21.5) | 2.9 (12.0) | 7.1 (19.3) |
| Smoking, (%) | | | |
| Never | 237 (15.4) | 916 (43.4) | 1153 (31.6) |
| Former | 1100 (71.5) | 921 (43.6) | 2021 (55.4) |
| Current | 201 (13.1) | 276 (13.1) | 477 (13.1) |
| Educational level, (%) | | | |
| Primary | 122 (7.9) | 293 (13.9) | 415 (11.4) |
| Low | 472 (30.7) | 1162 (55.0) | 1634 (44.8) |
| Intermediate | 602 (39.1) | 528 (25.0) | 1130 (31.0) |
| High | 342 (22.2) | 130 (6.2) | 472 (12.9) |
| Physical activity, hours/month | 68.7 (53.3) | 90.0 (56.7) | 81.1 (55.4) |
| Systolic blood pressure, mm/Hg | 147.0 (27.5) | 150.0 (28.0) | 148.5 (28.0) |
| Diastolic blood pressure, mm/Hg | 80.5 (15.0) | 79.0 (14.0) | 80.0 (14.5) |
| Cholesterol, mmol/L | 5.28 (1.23) | 5.84 (1.24) | 5.60 (1.28) |
| High-density lipoprotein cholesterol, mmol/L | 1.24 (0.41) | 1.51 (0.54) | 1.39 (0.52) |
| Diabetes, (%) | 133 (8.6) | 142 (6.7) | 275 (7.5) |
| Stroke, (%) | 25 (1.6) | 17 (0.8) | 42 (1.2) |
| *APOE*-ε4, (%) | 390 (26.5) | 527 (26.7) | 917 (26.6) |
| Total body bone mineral density , g/cm$^2$ | 1.20 (0.13) | 1.06 (0.14) | 1.12 (0.17) |
| Femoral neck bone mineral density, g/cm$^2$ | 0.92 (0.18) | 0.82 (0.17) | 0.86 (0.19) |
| Lumbar spine bone mineral density, g/cm$^2$ | 1.21 (0.27) | 1.04 (0.24) | 1.10 (0.28) |
| Trabecular bone score, mm$^{-1}$ | 1.33 (0.12) | 1.25 (0.14) | 1.28 (0.14) |

Definition of abbreviations: *APOE* = Apolipoprotein E. Data presented as mean (SD) or median (interquartile range). Proportions of missing data: alcohol intake (2.1%), *APOE* genotype (5.6%), body mass index (1.5%), diabetes (5.1%), education attainment (1.6%), HDL (1.8%), physical activity (3.8%), serum total cholesterol (1.8%), and systolic and diastolic blood pressure (0.2%).

Missing data were imputed using bayesian linear regression for continuous variables, logistic regression for binary variables, and polytomous logistic regression for categorical variables with more than two subgroups.

TABLE 2 Bone mineral density and the risk of incident dementia stratified by incremental epochs of follow-up time

| | 0~5 years | | 0~10 years | | Total follow-up | |
|---|---|---|---|---|---|---|
| | n/N | HR (95%CI) | n/N | HR (95%CI) | n/N | HR (95%CI) |
| Femoral neck bone mineral density | | | | | | |
| Highest tertile | 8/1195 | 1 | 49/1195 | 1 | 201/1195 | 1 |
| Medium tertile | 7/1194 | 0.85 (0.26, 2.73) | 67/1194 | 1.34 (0.91, 1.98) | 236/1194 | 1.16 (0.96, 1.42) |
| Lowest tertile | 16/1195 | 2.32 (0.84, 6.44) | 86/1195 | **2.03 (1.39, 2.96)** | 229/1195 | **1.26 (1.03, 1.54)** |
| Per SD decrease | 31/3584 | **2.13 (1.28, 3.57)** | 202/3584 | **1.43 (1.19, 1.72)** | 666/3584 | **1.12 (1.02;1.23)** |
| Lumbar spine bone mineral density | | | | | | |
| Highest tertile | 10/1203 | 1 | 64/1203 | 1 | 224/1203 | 1 |
| Medium tertile | 11/1202 | 1.09 (0.41, 2.91) | 67/1202 | 1.07 (0.74, 1.54) | 224/1202 | 0.96 (0.79, 1.17) |
| Lowest tertile | 12/1203 | 1.23 (0.47, 3.20) | 80/1203 | 1.27 (0.89, 1.80) | 233/1203 | 1.00 (0.82, 1.21) |
| Per SD decrease | 33/3608 | 1.04 (0.69, 1.56) | 211/3608 | 1.08 (0.93, 1.27) | 681/3608 | 0.97 (0.89;1.05) |
| Total body bone mineral density | | | | | | |
| Highest tertile | 12/1211 | 1 | 68/1211 | 1 | 227/1211 | 1 |
| Medium tertile | 6/1211 | 0.49 (0.16, 1.46) | 57/1211 | 0.85 (0.58, 1.24) | 227/1211 | 1.00 (0.83, 1.22) |
| Lowest tertile | 15/1211 | 1.00 (0.39, 2.56) | 90/1211 | **1.42 (1.01, 2.02)** | 232/1211 | 1.00 (0.82, 1.22) |
| Per SD decrease | 33/3633 | 1.27 (0.77, 2.08) | 215/3633 | **1.22 (1.00, 1.47)** | 686/3633 | 1.02 (0.92, 1.14) |
| Trabecular bone score | | | | | | |
| Highest tertile | 10/1191 | 1 | 59/1191 | 1 | 210/1191 | 1 |
| Medium tertile | 12/1191 | 2.47 (0.94, 6.52) | 74/1191 | **1.55 (1.08, 2.21)** | 226/1191 | 1.21 (0.99, 1.47) |
| Lowest tertile | 11/1191 | 2.04 (0.73, 5.68) | 77/1191 | **1.59 (1.11, 2.28)** | 236/1191 | 1.19 (0.98, 1.45) |
| Per SD decrease | 33/3573 | 1.37 (0.92, 2.04) | 210/3573 | 1.16 (1.00, 1.35) | 672/3573 | 1.04 (0.95;1.14) |

Definition of abbreviations: n = Cases, N = Total participants, *APOE* = Apolipoprotein E; CI = Confidence Interval; HR = Hazard Ratio; SD = Standard Deviation. Cox regressions were adjusted for age, sex, *APOE* genotype, education attainment, physical activity, smoking status, body mass index, systolic and diastolic blood pressure, total cholesterol levels, high-density lipoprotein cholesterol levels, and history of comorbidities (stroke and diabetes mellitus).

* follow-up time started after bone mineral density scans at baseline.

* The tertile categories of bone mineral density were derived by generating tertiles from the residuals of linear regression models adjusted for age (continuously) and sex. The highest tertile as the reference group.

Bold font corresponds to significant P-value threshold.

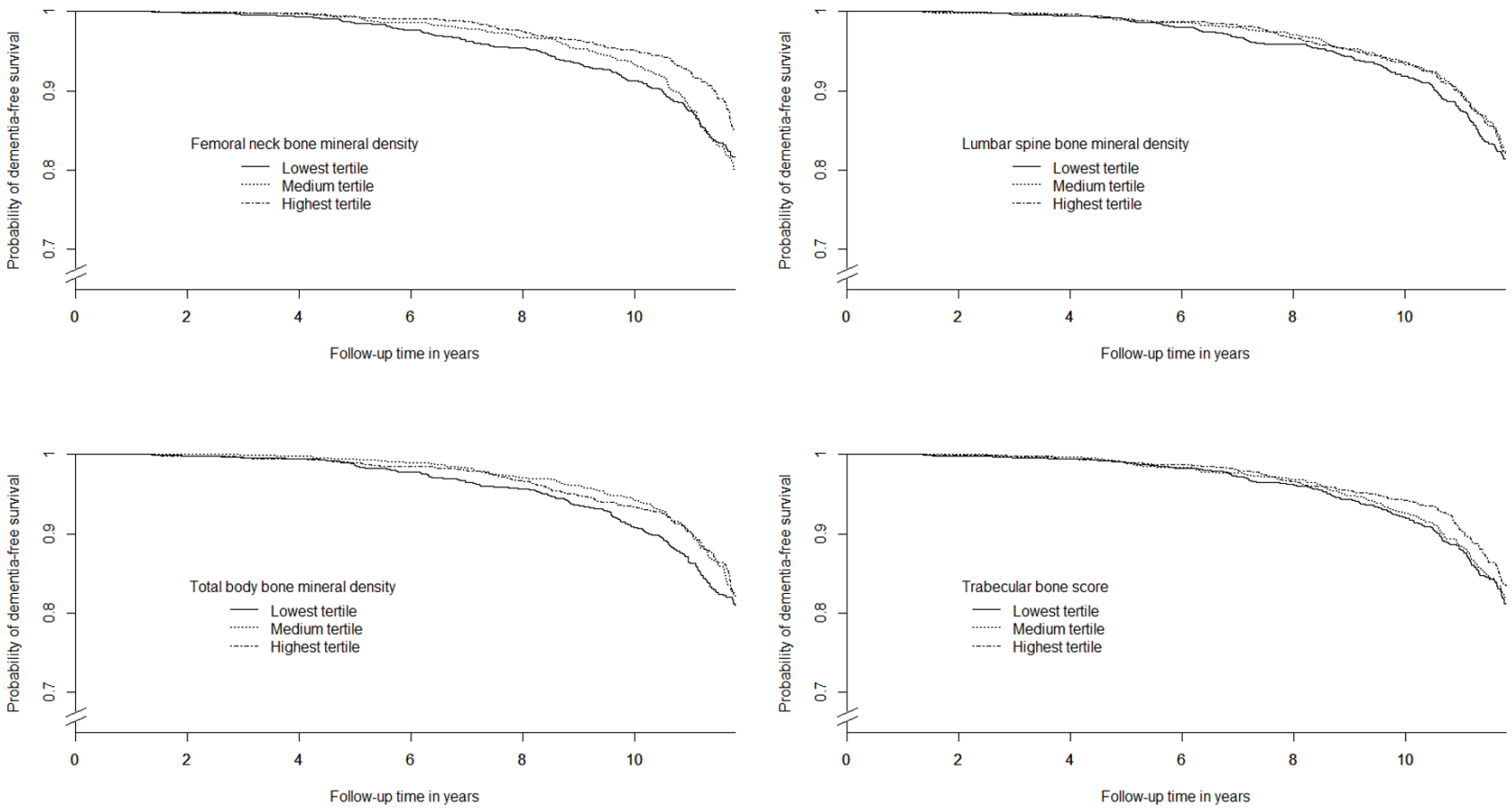



**FIGURE 2** Kaplan-Meier curves of dementia-free survival at different levels of bone mineral density at each site.

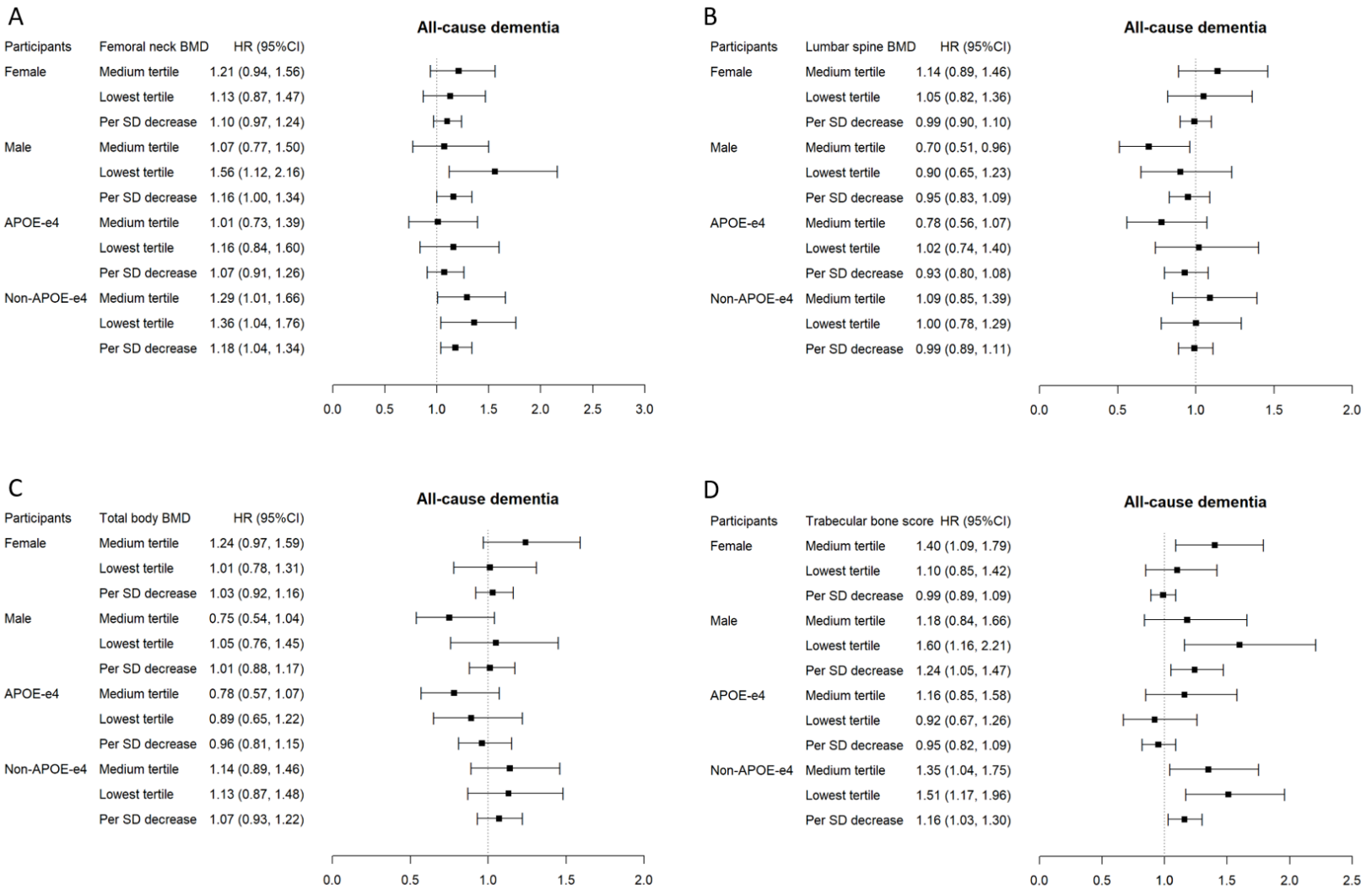



**FIGURE 3** Associations of low bone mineral density of the total body (A), the femoral neck (B), the lumbar spine (C), and trabecular bone scores with the risk of all-cause dementia, stratified by sex and *APOE*-ε4 allele carriership. *APOE* = Apolipoprotein E; BMD =

Bone Mineral Density; HR = Hazard Ratio. Participants in the highest tertile of bone mineral density were regarded as the reference group (hidden). Estimated HRs were obtained after adjustment of (if applicable) age, sex, *APOE* genotype, education attainment, physical activity, smoking status, body mass index, systolic and diastolic blood pressure, total cholesterol levels, high-density lipoprotein cholesterol levels and history of comorbidities (stroke and diabetes mellitus).

*The tertile categories of bone mineral density were derived by generating tertiles from the residuals of linear regression models adjusted for age (continuously) and sex. The highest tertile as the reference group.

eTable 1. Bone mineral density and the risk of incident Alzheimer's disease stratified by incremental epochs of follow-up time

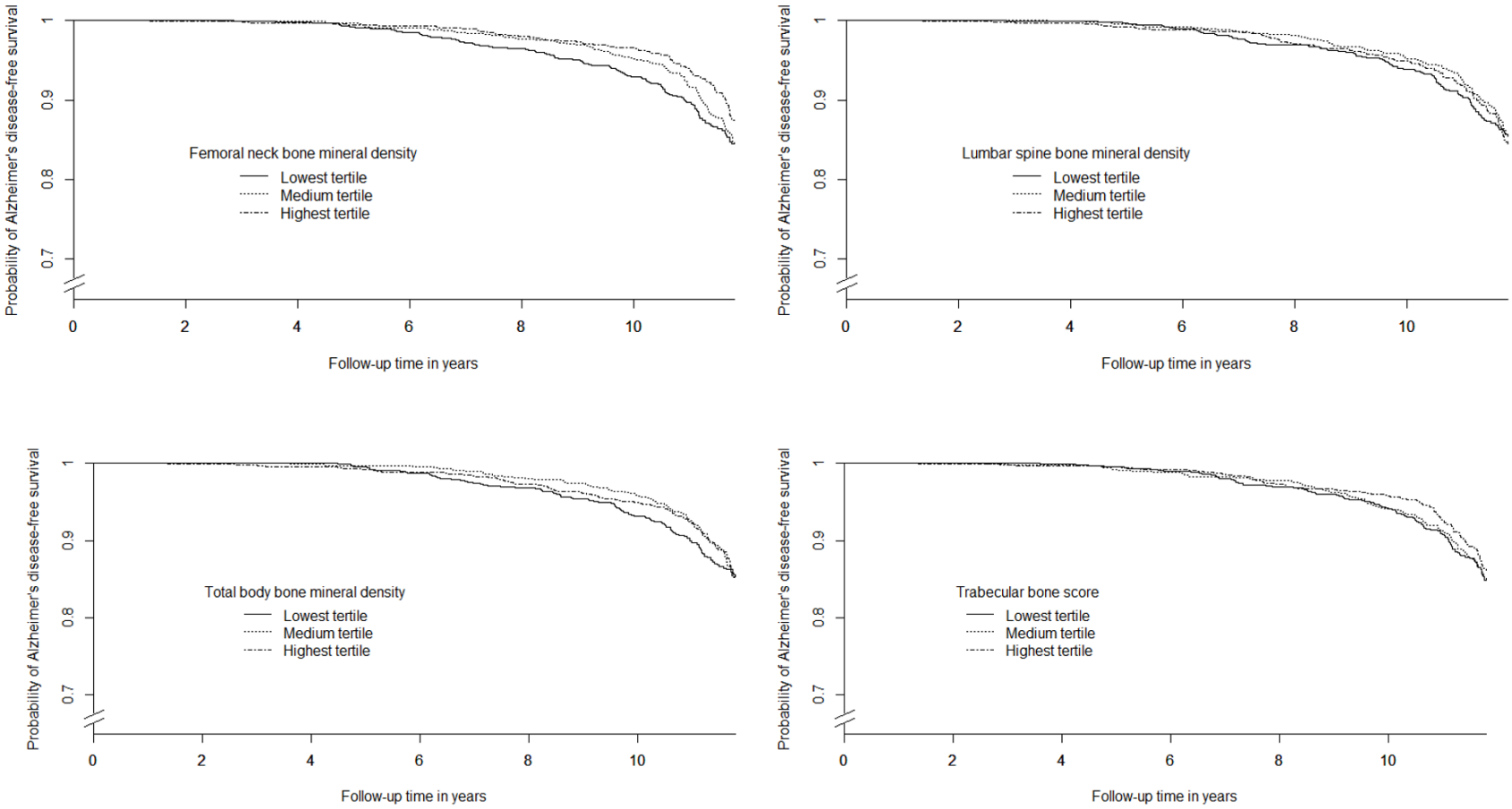
| | 0~5 years | | 0~10 years | | Total follow-up | |
|---|---|---|---|---|---|---|
| | n/N | HR (95%CI) | n/N | HR (95%CI) | n/N | HR (95%CI) |
| Femoral neck bone mineral density | | | | | | |
| Highest tertile | 6//1143 | 1 | 33/1143 | 1 | 151/1143 | 1 |
| Medium tertile | 3/1143 | 0.83 (0.23, 2.96) | 45/1143 | 1.41 (0.87, 2.27) | 178/1143 | 1.13 (0.90, 1.42) |
| Lowest tertile | 8/1144 | 2.32 (0.81, 6.63) | 65/1144 | **2.30 (1.45, 3.64)** | 183/1144 | **1.32 (1.05, 1.66)** |
| Per SD decrease | 17/3429 | 1.85 (0.93, 3.70) | 143/3429 | **1.52 (1.20, 1.92)** | 512/3429 | **1.14 (1.02;1.28)** |
| Lumbar spine bone mineral density | | | | | | |
| Highest tertile | 9/1150 | 1 | 48/1150 | 1 | 171/1150 | 1 |
| Medium tertile | 5/1150 | 0.54 (0.17, 1.71) | 45/1150 | 0.94 (0.61, 1.45) | 174/1150 | 0.99 (0.79, 1.24) |
| Lowest tertile | 3/1151 | 0.31 (0.09, 1.13) | 57/1151 | 1.14 (0.76, 1.72) | 179/1151 | 0.97 (0.78, 1.21) |
| Per SD decrease | 17/3451 | 0.72 (0.48, 1.10) | 150/3451 | 1.08 (0.89, 1.30) | 524/3451 | 0.97 (0.88;1.08) |
| Total body bone mineral density | | | | | | |
| Highest tertile | 9/1158 | 1 | 50/1158 | 1 | 170/1158 | 1 |
| Medium tertile | 3/1158 | 0.37 (0.10, 1.33) | 39/1158 | 0.78 (0.50, 1.23) | 182/1158 | 1.06 (0.85, 1.32) |
| Lowest tertile | 5/1158 | **0.30 (0.10, 0.97)** | 63/1158 | 1.29 (0.85, 1.95) | 175/1158 | 0.97 (0.78, 1.22) |
| Per SD decrease | 17/3474 | 0.74 (0.47, 1.16) | 152/3474 | 1.20 (0.95, 1.52) | 527/3474 | 1.00 (0.88;1.12) |
| Trabecular bone score | | | | | | |
| Highest tertile | 5/1139 | 1 | 41/1139 | 1 | 161/1139 | 1 |
| Medium tertile | 8/1139 | 4.88 (0.99, 23.93) | 55/1139 | **1.66 (1.08, 2.54)** | 173/1139 | 1.21 (0.97, 1.52) |
| Lowest tertile | 4/1139 | 2.45 (0.42, 14.17) | 53/1139 | **1.55 (1.00, 2.39)** | 182/1139 | 1.16 (0.93, 1.45) |
| Per SD decrease | 17/3417 | 1.22 (0.66, 2.27) | 149/3417 | 1.15 (0.96, 1.39) | 516/3417 | 1.02 (0.93;1.14) |

Definition of abbreviations: n = Cases, N = Total participants, *APOE* = Apolipoprotein E; CI = Confidence Interval; HR = Hazard Ratio; SD = Standard Deviation. Cox regressions were adjusted for age, sex, *APOE* genotype, education attainment, physical activity, smoking status, body mass index, systolic and diastolic blood pressure, total cholesterol levels, high-density lipoprotein cholesterol levels, and history of comorbidities (stroke and diabetes mellitus).

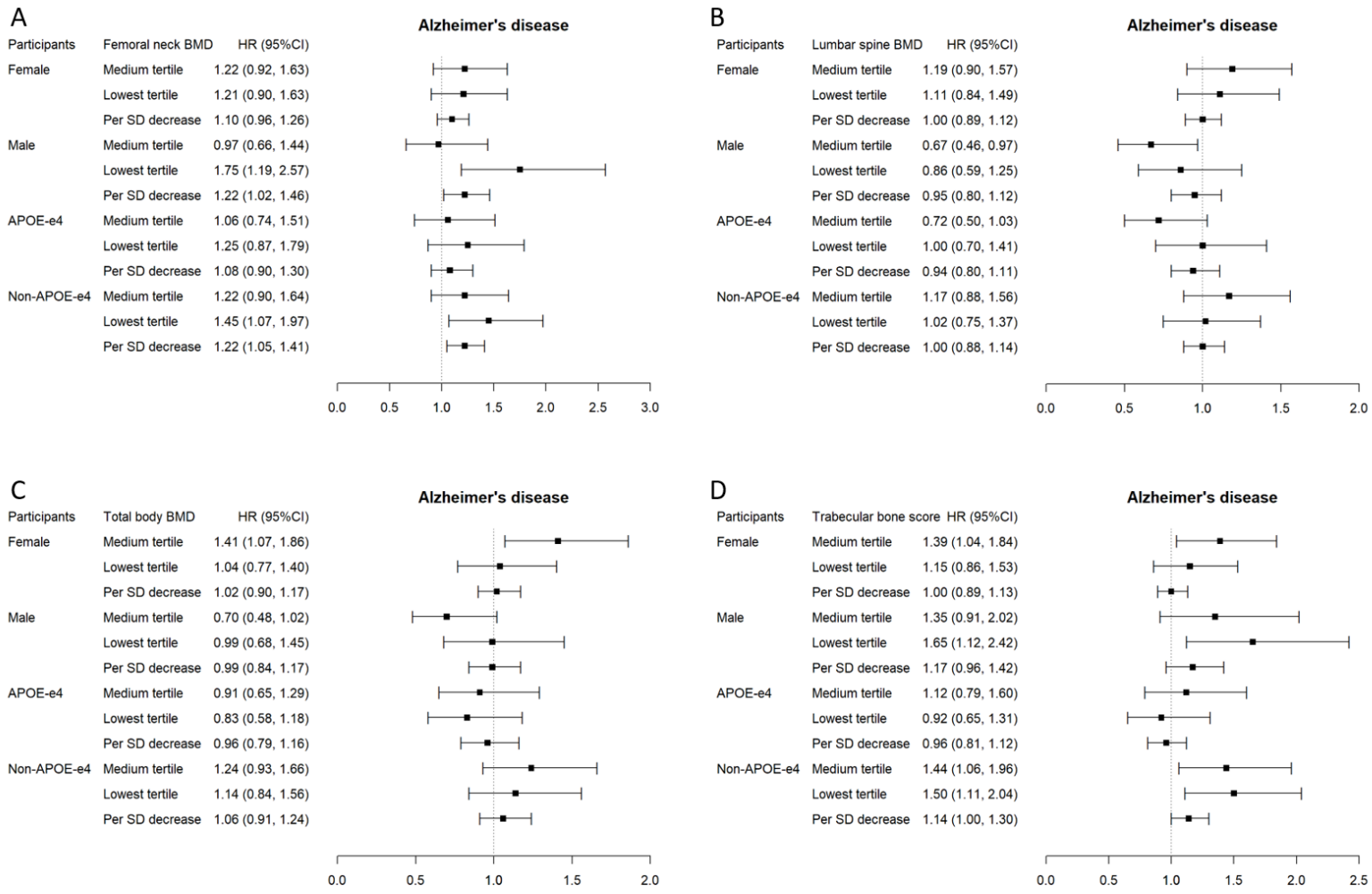
* follow-up time started after bone mineral density scans at baseline.

* The tertile categories of bone mineral density were derived by generating tertiles from the residuals of linear regression models adjusted for age (continuously) and sex. The highest tertile as the reference group.

Bold font corresponds to significant P-value threshold.




eFigure 1. Kaplan-Meier curves of Alzheimer's disease-free survival at different levels of bone mineral density at each site.




**eFigure 2.** Associations of low bone mineral density of total body (A), the femoral neck (B), the lumbar spine (C), and trabecular bone scores with risk of Alzheimer's disease, stratified by sex and *APOE*-ε4 allele carriership. *APOE* = Apolipoprotein E; BMD = Bone

Mineral Density; HR = Hazard Ratio. Participants in the highest tertile of bone mineral density were regarded as the reference group (hidden). Estimated HRs were obtained after adjustment of (if applicable) age, sex, *APOE* genotype, education attainment, physical activity, smoking status, body mass index, systolic and diastolic blood pressure, total cholesterol levels, high-density lipoprotein cholesterol levels, and history of comorbidities (stroke and diabetes mellitus).

* The tertile categories of bone mineral density were derived by generating tertiles from the residuals of linear regression models adjusted for age (continuously) and sex. The highest tertile as the reference group.

# Chapter 6 General Discussion

In this thesis, I discuss the role of systemic function impairment in neurodegeneration, studied from a population-based prospective. In this chapter, I summarize the key findings while also highlighting several methodological considerations, implications, and future perspectives.

## MAIN FINDINGS

In this thesis, I drew the link between the abnormality of other organ systems, neurodegenerative diseases, and related brain outcomes.

### Pulmonary System and Neurodegeneration

In Chapter 2, I examined whether lung function impairment increases neurodegeneration using respiratory tests and a clinical diagnosis of chronic pulmonary disease. In Chapter 2.1 I reported that a decline in lung function was linked to a higher risk of dementia. In Chapter 2.2 I investigated whether lung function impairment is related to cognitive performance and the presence of vascular brain lesions. I demonstrated that people with more severe COPD or limited lung function had worse overall cognitive performance and a larger prevalence of lacunar infarcts.

### Hepatic System and Neurodegeneration

In Chapter 3, I examined the relationship between the prevalence of neurodegenerative disorders and fatty liver disease (FLD). I focused on non-alcoholic fatty liver disease (NAFLD) in Chapter 3.1 and discovered that neither NAFLD nor fibrosis was linked to an increased risk of dementia incidentally, nor was NAFLD linked to poorer cognitive function. A non-significant relationship between FLD and parkinsonism or Parkinson's disease was found in Chapter 3.2. I used both abdominal ultrasound and the fatty liver index to diagnose liver steatosis, in contrast to prior

research [169, 297], and found a negative association between fatty liver disease and neurodegenerative disorders.

**Cardiac System and Neurodegeneration**

In Chapter 4 I investigated how cardiac biomarkers impact changes in cognition and structural brain alteration, as well as the incidence of Parkinsonism and Parkinson's disease. In Chapter 4.1 I used longitudinal data to examine the effect of NT-proBNP on the trajectories of cognition and brain imaging markers with repeated measurements. The results demonstrated that greater baseline NT-proBNP levels were related to a faster deterioration in cognition but not to structural brain alterations. In Chapter 4.2 the perspective on neurodegeneration changed from estimating trajectories of brain markers to the incidence of neurodegenerative diseases, specifically Parkinsonism and Parkinson's disease. I found that high levels of cardiac biomarkers were not related to an increased risk of Parkinson's disease. These findings could contribute to the body of knowledge regarding the relationship between cardiac biomarkers and parkinsonism and Parkinson's disease, given the dearth of prospective studies on this subject.

**Musculoskeletal System and Neurodegeneration**

In Chapter 5 I determined how dementia risk was affected by bone mineral density at various skeletal regions in community-dwelling older adults. In Chapter 5.1 I stratified analyses by incremental epochs of follow-up time and found that participants with low femoral neck, low total body bone mineral density, or low trabecular bone score were more likely to develop dementia. Similar findings temporally linking bone mineral density to dementia risk were also reported by

previous prospective population-based studies [256, 257], this indicated that low bone mineral density might be a concurrent sign during the cognitive decline preceding the dementia onset.

## METHODLOGICAL CONSIDERATIONS

The pertinent parts of this thesis have outlined the strengths and limitations of each investigation. Thus, I further discuss more general underpinnings on how to interpret current findings in this thesis.

### The Scope of Systemic Function Impairment

This thesis defines systemic dysfunction as a framework that includes a variety of comorbidities, course of clinical conditions, and severity levels of dysfunction assessed by markers or indexes for specific peripheral organs. Clinically speaking, a disease is an abnormal condition that describes organ dysfunction or structural abnormalities. Most contemporary definitions of diseases or disorders combine the concepts of symptoms, imaging or biopsy evidence of abnormality, and/or deviating biomarkers or physical tests due to clinical complexity. However, in some instances, not all of the aforementioned three factors are necessary for the diagnosis of a disease, particularly for some treatable illnesses like non-alcoholic fatty liver disease (NAFLD). In Chapter 3, NAFLD was diagnosed using a fatty liver index or abdominal ultrasound scan, without any signs of symptoms. Most NAFLD patients do not encounter evident liver dysfunction itself or complications and will recover after 5-10% weight reduction [160, 298]. The association between NAFLD and neurodegeneration was far more complicated than we expected. This relationship is also time-dependent, particularly when it comes to the dynamic change in hepatic steatosis that occurs after a lifestyle change. Besides, the course and severity of a disorder are also two critical determinants

when considering the impact on brain health. In Chapter 2, preserved ratio impaired spirometry (PRISm), as an intermediate phase between normal spirometry and COPD, is a heterogeneous and fluctuating condition but is always undiagnosed in clinical practice [89]. Longitudinal studies are still necessary to evaluate the impact of PRISm and the severity of COPD on neurodegenerative disorders.

Apart from the presence of disease diagnosis, we also used the clinical diagnosis markers as proxies to quantify specific sorts of systemic function impairment, such as using NT-proBNP to represent cardiac dysfunction as presented in Chapter 4. How to systemically analyze the function of organs will determine how much intrinsic knowledge may be unlocked to target the accuracy of functional loss. For instance, a thorough evaluation of cardiac function necessitates both anatomic and physiological assessment of the heart's structure and its physiological functioning [299]. Specific cardiac function-associated markers in plasma exhibit unique advantages in patient screening in population-based settings due to lower cost and a lower likelihood of measurement error than complex tests carried out in a specialized setting, despite reflecting less information than cardiac imaging [300].

**Bias**

Bias as a systemic error, in contrast to random variation, could impact the associations, undermine the robustness of conclusions, and weaken the generalizability of findings. I will discuss how three main epidemiological biases, including selection bias, information bias, and confounding bias, impact the study findings of this thesis.

Selection bias originates from the included participants for analyses systemically different from or not representative of the source population. Generally speaking, a higher response rate should

indicate a lower possibility of selection bias or a smaller effect on the association caused by the non-randomized selection of participants. Although there is overall a high response rate of over 70% within the Rotterdam Study, selection bias at baseline might still inevitably exist, because it is known that healthier people are more willing or highly likely to visit the medical center for physical examinations and interviews. As such, it is plausible to infer that participants with better systemic function or cognition are more possible to participate in various function tests and brain imaging scans, especially in multiple rounds of visits for estimating trajectories of outcomes. Besides, a loss to follow-up occurs when a participant drops out from the cohort, which makes it impossible to assess the status of interest. The participant is then censored when performing analyses. If the rate of dropout varies depending on certain participant characteristics, such as when individuals of older age or with worse health status (difficulty in motion, severe pulmonary dysfunction, and et al) are more likely to drop out than those with better status, this is referred to as differential loss to follow-up. In practice, the follow-up on dementia, Parkinson's disease, and related clinical outcomes was nearly complete in the Rotterdam Study. In addition, the average baseline age of about 70 years and the European ethnicity of participants restrict the generalizability of findings to non-Caucasians and younger populations (see also Chapter 4.1).

Information bias is caused by imprecise measurement or misclassification of exposures, outcomes, or co-variables. Within the Rotterdam Study, multiple efforts have been put into constructing standardized procedures and training personnel for data collection, management, manipulation, and interpretation. Nevertheless, information bias could be not completely precluded even though all the above actions have been put into execution. The misclassification could occur more or less within the exposure diagnosis or measurement. For instance, in Chapter 3 I investigated fatty liver disease about neurodegenerative diseases. Hepatic steatosis was

diagnosed with ultrasound, as well as fatty liver index, an algorithm with cutoff values of $< 30$ and $\geq 60$ to rule out or rule in hepatic steatosis, respectively, among which ultrasound presents a higher accuracy of detecting hepatic fat accumulation than fatty liver index [301]. Pragmatically, liver biopsy is the clinically gold standard for the diagnosis of hepatic steatosis than other non-invasive methods. This suggests participants with undetected fatty liver disease could be misclassified as an unexposed group, which could affect the effect magnitude of the association between fatty liver disease and neurodegenerative diseases. Information bias may differently affect the associations between studies.

Confounding bias occurs when mixing the effect of the exposure of interest with extraneous risk factors of the outcome, that is to say, confounding arises when the exposure and outcome share one or more causes. Related to this, a confounder is any variable that can be used to help eliminate confounding [302]. Controlling for confounders is essential for reliable causal inference, and a key step is confounder selection. An appropriate selection of confounders requires adequate knowledge of a complete causal diagram and the availability of confounder measurement [303]. In this thesis, confounders were controlled when they were common causes of the exposure and the outcome based on previous literature and biological plausibility. However, it has been pointed out that in certain circumstances this could introduce unexpected bias, such as residual confounding. Residual confounding would arise when there is a missing adjustment for unknown confounders based on current evidence. For example, in Chapter 5.1 I studied the association between cardiac biomarkers and the risk of Parkinson's disease, in which only age, sex, smoking status, and education levels were incorporated for calculating effect estimation. Other risk factors, such as obesity, diabetes, alcohol consumption, and physical inactivity are the traditional causes of cardiac dysfunction or triggering release of cardiac biomarkers but show inconsistent relations with Parkinson's disease

between different studies with heterogeneity or limitations. Taken together, it is possible that the majority of associations in this thesis were to some degree influenced by confounding bias. Even though within the Rotterdam Study there is a large number of co-variables available for control, it remains impossible to make a complete selection of confounders or even to adjust for all confounders in analyses.

Precision is a description of random errors and increasing sample size generally increases precision. Small sample sizes are also associated with a decreased likelihood of initially identifying real effects. This raises concerns about the precision of findings in a large portion of dementia research. In addition, underpowered studies may give rise to publication bias due to selective reporting of statistically significant results. The Rotterdam Study serves as the data source for dementia study in the majority of the investigations in this thesis. The large sample size from this population-based cohort gives it a better opportunity (more power) to find real effects. However, there are limited cases of incident Parkinson's disease due to an insufficient sample size. For example, in Chapter 3.2, I included 9,364 participants at baseline, and among them, only 74 individuals develop Parkinson's disease over a median follow-up of 11 years. This lack of precision may have unpowered us to detect small effects.

**Reverse Causality**

Reverse causality refers to the opposite direction of causality, changing from the exposure causing the occurrence of the outcome to the "outcome" resulting in changes in the "exposure". Before the clinical onset of dementia, patients in the prediagnostic phase experience a period of accelerated cognitive decline, accompanied by pathological changes in the brain [83]. In this scenario, the loss of cognition preceding dementia inevitably influences the quality of life among the elderly by modifying self-care ability and altering lifestyles, such as physical activity, nutrient balance, social

interaction, reading, et al.[304], which might, in turn, impacts a change in the exposure of primary interest. In this scenario, the temporal relation is causal but reverse, and the association can be thought of as confounded by cognitive decline or neuropathology.

In Chapter 5.1 I investigated the bone mineral density and dementia risk. The reverse causality was examined via stratifying Cox models by incremental epochs of follow-up time to study how the risk of incident dementia changed over follow-up duration (with restricted epochs e.g., baseline to 5 years, baseline to 10 years, baseline to over 10 years). The risk of dementia disappearing with follow-up time extending implies prodromal or prediagnostic disease-disrupting bone mineral density at baseline. Importantly, when performing stratification on follow-up time, it is always challenging to keep a balance between the statistical power for detecting significant associations and sensitivity for testing reverse causality. Within the too short term of the follow-up period, a limited number of incident cases could restrict or impair the statistical power of the association. This analytic approach shows a temporal relation and indicates reverse causality, but could not preclude the possibility of a causality. With follow-up time increasing, the disappearance of risk or the causality could be attributed to several aspects, including 1) The pathogenesis of dementia comprises complex biological pathways, and the journey to disease onset contains multistage processes as found in a prospective population-based cohort [305]. It is, therefore, plausible to infer that the exposure-dementia link should be both pathway- and stage-dependent, reflected by risk changes with different follow-up windows. 2) Accumulative effect of harmful exposures could increase the risk of mortality, especially with longer follow-up duration, and thus death as a competing risk may also affect the associations. 3) The exposure status may regress or deteriorate over time and this may introduce information bias when estimating the association. In Chapter 3, I found that over the whole follow-up, NAFLD did not increase dementia risk. Conversely, within

the first five years of follow-up, NAFLD exerts a protective effect against dementia, implying NAFLD regression before dementia occurrence. In this case, long-term trajectories of exposure status, instead of a single static measure at baseline, could unveil a more accurate and dynamic estimation of disease risk.

## IMPLICATIONS AND FUTURE RESEARCH

Various directions in pathophysiological mechanisms and methodological improvements can be highlighted for future investigations in light of the findings in this thesis. First, other systemic comorbidities, i.e. olfactory deficit, osteoarthritis, and gastritis, have received little attention about their effect on dementia risk. Future studies could explore the effects of other ignored comorbidities, either in isolation or in conjunction with known comorbidities, on dementia. Second, Comorbidities are common among patients of Parkinson's disease, but it remains not well explored regarding the direction of potential causality between comorbidities and Parkinson's disease. Third, multiorgan MRI enables us to comprehensively assess structural and functional changes in brain and other systemic organs. Fourth, it remains to be ascertained whether the systemic dysfunction is causally related to incident dementia. Fifth, it is still important to investigate how determinants change over time in dementia, as in the real world, determinants are likely modified by various influences. I will discuss each of these proposed directions in the following paragraphs.

### Other Systemic Comorbidities and Neurodegeneration

To gain an intact insight into pathological mechanisms linking systemic dysfunction to neurodegeneration, more attention should also be paid to less well-studied systemic comorbidities when considering their potential for dementia occurrence. The prevalence of smell sense loss is

around 5%, as people age, the proportion substantially increases to 13.9% in individuals aged > 65 years [306]. In recent years, a significant association has been reported between olfactory impairment and the risks of cognitive deficits and dementia [307-310]. However, the magnitude of evidence could be undermined by small sample sizes and less-optimal adjustment of confounding. For instance, airborne lead pollution should be an important confounder and plays a role in these associations [311]. Higher cumulative exposure to lead, as a widely known neurotoxin, is a significant risk factor for cognitive impairment, structural brain alteration, and deposition of neuropathological proteins [312-314]. Apart from the olfactory system, osteoarthritis, as a common joint disorder among the elderly, receives extra attention regarding its role in brain health [315, 316]. Recent studies suggest a faster Aβ accumulation and a higher level of tau deposition among patients with osteoarthritis or in osteoporotic bone tissues[280, 317]. Few cohort studies reported an association between osteoarthritis and a higher risk of dementia and brain atrophy [318, 319]. Similarly, the causality between the two disorders was limited by residual confounding due to missing adjustments for key confounders, including bone mineral density, body mass index, and *APOE* genotype. An accumulative body of evidence indicates that the deficiency of vitamin B12 and anemia might be two risk factors for cognitive decline [320-323]. Gastritis might lead to dementia occurrence regarding the fact that gastritis could impede the intake of micronutrients, including vitamin B12 and iron, and further cause iron deficiency anemia [324-326]. It is plausible to infer gastritis as a possible risk factor for dementia [327], but more prospective evidence is warranted. Moreover, the (causal-) roles of skin on neurodegeneration have gained increasing attention but remained understudied [328, 329]. Future studies could also explore the mixed effects of multiple comorbidities in conjunction, on dementia. Most of the previous studies mainly focus on how single comorbidity impacts brain health. As people age, it is more likely to suffer from multiple

comorbid conditions [8], and it is commonly observed that dementia patients are diagnosed with multiple comorbidities [9, 10]. It will be, therefore, more clinically reasonable and valuable to study how various comorbidities together contribute to dementia development.

Future research should also look into the association between systemic function impairment and the onset of Parkinson's disease (PD) and secondary parkinsonism. Previous epidemiological studies [330-332] showed distinct burdens of various comorbidities amongst PD patients, and the most frequent comorbidities were hypertension, diabetes, depression, atrial fibrillation, arthritis, et al. Although the prevalence of some chronic diseases in PD patients is similar to the general population, evidence from meta-analyses suggests that patients with these comorbidities, such as hypertension [333] and diabetes [334, 335], have a higher risk of developing PD. The links between other comorbidities and PD, however, have not been well investigated. For example, COPD was associated with an increased risk of dementia and mild cognitive impairment, and low respiratory parameters ($FEV_1$% predicted and/or FVC% predicted) were indicated as risk factors for dementia, stroke, poor cognition, and vascular brain lesions [48, 90, 336, 337]. Given the fact that dementia and PD share biological processes, it is important to evaluate the association between lung function impairment and PD. Only one prospective study has, to date, explored the potential connection between COPD and PD risk [338]. The absence of quantitative respiratory measures and the failure to account for smoking, however, hampered the results. Additionally, underlying mechanisms suggest that PD may contribute to cardiovascular conditions like atrial fibrillation, but these connections are not well established, and results from earlier studies varied widely [339].

**Multiorgan MRI**

Multiorgan MRI should be used to provide objective assessments of an organ status with more detailed information than the single-task MRI mainly focusing only on one (or a few) traits. For instance, in Chapter 4.1 I studied the impact of plasma NT-proBNP levels on cognition and global brain structure. Limited information about cardiac and brain structure and/or function precluded us from obtaining an overall picture of heart-brain interplay. Although NT-proBNP is regarded as a well-established diagnostic marker of heart failure, it alone could not depict an overview of heart structure and how cardiac structural changes contribute to brain dysfunction. Similarly, the main focus on global brain structure also ignores the other aspects of the brain, such as regional or tract-based structural connectivity, functional activity, et al. Comprehensive MRI modalities allow us to identify detailed information about specific organs' structure and function, yielding better insights into pathological alterations in organs. Recently, a large-scale study quantified the heart-brain interaction using multi-organ MRI-derived traits (82 cardiac and aortic traits and 458 brain traits) among >40,000 individuals [340]. Heart MRI traits were observed associated with multiple brain modalities, including specific brain regions, white matter tracts, and functional networks. The findings further underscored the high values of multiorgan MRI when investigating how systemic dysfunction comprehensively relates to brain health.

**Causality**

Several methodological points should be clarified for strengthening causal inference. To ideally test the causality between determinants and outcome, a randomized controlled trial is the first choice for this purpose, but this is impractical and unethical regarding systemic dysfunction as an intervention in humans. There are still some study designs that help draw a causal conclusion, though.

First, Mendelian randomization (MR), a causal inference methodology that uses genetic tools to replace exposures, has been widely developed and used to research causal relationships of interest to overcome the inherent constraints of observational studies. [341]. The MR design based on large GWAS studies could remove confounding bias because alleles are randomly allocated at conception, and it also avoids reverse causation bias as disease or event of interest cannot affect genotype [341]. Amongst the previous studies, few of them have assessed causal associations of systemic dysfunction with dementia risk and relevant clinical manifestations using the MR design.

Second, regarding a long period of dementia development, investigation of trajectories of brain structure or cognition could help plausible inferences on a temporal link between systemic dysfunction and markers of brain aging or cognition in longitudinal studies with repeated measurements. A more comprehensive insight into cerebral pathophysiology would benefit from clarifying the causality of the associations with dementia.

Finally, the diagnosis of Alzheimer's disease or dementia could be clarified by its underlying pathological formation of toxic proteins, such as amyloid-β plaques and neurofibrillary tau tangle in the brain, accompanied by progressive neuronal and synaptic loss [342, 343]. Particularly, in the preclinical phase before the diagnosis onset, an individual without or with only subtle symptoms of the disease might be detected earlier with an assessment of abnormal levels of the above biomarkers [344, 345]. The association between systemic dysfunction and changes in dementia-related biomarkers might assist in whether systemic dysfunction causally contributes to the onset of dementia. However, keep in mind that dementia-associated biomarkers may also be strongly associated with other chronic conditions, such as cardiovascular diseases, cancer, and multiple sclerosis [346-349]. It is therefore pertinent to select the best dementia-specific candidates of biomarkers for such diagnoses as the outcome of interest.

### Dynamic Effect

The time-dependent effect of specific risk factors should not be ignored when estimating dementia risk [350], that is to say, determinants would preferably be studied throughout their life course. Compared with a single measurement, long-term trajectories of systemic function may serve as a better indicator of later dementia occurrence. And these trajectories are beneficial for capturing the dynamic change of a function over time and visualizing the direction and size of variability. Ignorance of the changes in systemic function could not help map the dynamic effect on dementia risk across the long-term life course. Amounting evidence indicated that the patterns of some disorders or specific organs' function trajectories could also be complicated by alteration in lifestyle, adherence to medication, and/or growing age, which also gets involved in modifying dementia risk. NAFLD, as a classical example of reversible metabolic syndrome, could regress after weight loss through lifestyle modification [351, 352]. Recent evidence has indicated that hepatic steatosis can be reduced with weight loss of 3% to 5% and NAFLD regression occurs after 5-10% weight reduction [160, 298, 353]. After NAFLD diagnosis, over 50% of patients would experience above 5% weight changes, within 2 years and these changes usually persist for a while [354]. The relationship between NAFLD and dementia in this scenario should be dynamic rather than constant over time, and long-term trajectories of NAFLD status or hepatic steatosis may help redefine the association.

### Clinical Implications

Several implications for clinical practice should be considered based on the findings in this thesis. Increased awareness towards possible screening or testing systemic health status (i.e. lung function, cardiac function, bone health) could potentially be beneficial for identifying patients at high risk

of dementia or cognitive impairment. This is feasible, as most of the assessments are physically and non-invasive methods widely accepted in clinical settings. Additionally, even though there isn't any causal evidence from randomized controlled trials or Mendelian randomization just yet, systemic function data could help develop better neurodegenerative disease prediction tools or models. Paying more attention to cross-talk between systemic function and PD could potentially lead to potentially more insights into the etiology of PD and relevant recommendations for prevention.

Another main purpose of observational studies is to provide causative evidence for selecting the best medication candidates to prevent or slow down the development of neurodegeneration. Based on findings of this thesis, among medications for treating chronic pulmonary diseases, cardiac dysfunction and low bone mineral density, there are should be potential candidates for dementia clinical trials performed in patients with corresponding systemic function impairment. Compared with developing new drugs to cure dementia, screening an effective candidate from widely used medications would inevitably more economically effective and safe as all possible side effects are well known.

## CONCLUDING REMARKS

To conclude, this thesis provides an array of insights into the associations between systemic function impairment and neurodegeneration. In general, patients with systemic dysfunction could suffer from accelerated cognition decline and have an increased risk of dementia at a population level. I challenge future studies to investigate causality and provide biological insight into the link between these two disorders. This evidence could serve as a rationale for prevention and

intervention strategies. In addition, the study is scarce on how systemic function impairment relates to the development of Parkinson's disease, and more attention is warranted.

# Chapter 7 Summary/Samenvatting

Summary

As people age, the elderly experience a progressive loss of physiological function and structural alterations in multiple organs. Co-occurring comorbidities have been commonly observed in patients with dementia or Parkinson's disease, and evidence from epidemiological studies has already demonstrated the association between systemic dysfunction and a higher risk of dementia development. However, it remains unexplored about the roles of other understudied systemic function impairments in these brain disorders. A better understanding of the association between systemic malfunction, cognition, and brain abnormalities would allow us to develop a multi-system strategy for neurodegeneration prevention. This thesis's overarching goal is to better understand the relationship between systemic dysfunction and neurodegenerative disorders, notably dementia and Parkinson's disease, in middle-aged and older persons.

In **Chapter 2**, I examined whether lung function impairment increases neurodegeneration using respiratory tests and a clinical diagnosis of chronic pulmonary disease. In **Chapter 2.1** I reported that a decline in lung function was linked to a higher risk of dementia. In **Chapter 2.2** I investigated whether lung function impairment is related to cognitive performance and the presence of vascular brain lesions. I demonstrated that people with more severe COPD or limited lung function had worse overall cognitive performance and a larger prevalence of lacunar infarcts.

In **Chapter 3**, I examined the relationship between the prevalence of neurodegenerative disorders and fatty liver disease (FLD). I focused on non-alcoholic fatty liver disease (NAFLD) in **Chapter 3.1** and discovered that neither NAFLD nor fibrosis was linked to an increased risk of dementia incidentally, nor was NAFLD linked to poorer cognitive function. A non-significant relationship between FLD and parkinsonism or Parkinson's disease was found in **Chapter 3.2**. I used both

abdominal ultrasound and the fatty liver index to diagnose liver steatosis, and found a negative association between fatty liver disease and neurodegenerative disorders.

In **Chapter 4** I investigated how cardiac biomarkers impact changes in cognition and structural brain alteration, as well as the incidence of Parkinsonism and Parkinson's disease. In **Chapter 4.1** I used longitudinal data to examine the effect of NT-proBNP on the trajectories of cognition and brain imaging markers with repeated measurements. The results demonstrated that greater baseline NT-proBNP levels were related to a faster deterioration in cognition but not to structural brain alterations. In **Chapter 4.2** the perspective on neurodegeneration changed from estimating trajectories of brain markers to the incidence of neurodegenerative diseases, specifically Parkinsonism and Parkinson's disease. I found that high levels of cardiac biomarkers were not related to an increased risk of Parkinson's disease. These findings could contribute to the body of knowledge regarding the relationship between cardiac biomarkers and parkinsonism and Parkinson's disease, given the dearth of prospective studies on this subject.

In **Chapter 5** I determined how dementia risk was affected by bone mineral density at various skeletal regions in community-dwelling older adults. In **Chapter 5.1** I stratified analyses by incremental epochs of follow-up time and found that participants with low femoral neck, low total body bone mineral density, or low trabecular bone score were more likely to develop dementia. Our findings temporally linking bone mineral density to dementia risk indicated that low bone mineral density might be a concurrent sign during the cognitive decline preceding the dementia onset.

Lastly, in **Chapter 6** I discussed the main findings of this thesis, methodological considerations, implications, and future research. As for methodological perspectives, I discussed the framework of systemic function impairment. Additionally, I discussed the possibility and impact of bias (confounding bias, information bias, and selection bias), as well as the issue of reverse causation, in the studies of this thesis. Lastly, I proposed the clinical implications and proffer the potential directions for future studies.

# Samenvatting

Naarmate mensen ouder worden, ervaren ouderen een progressief verlies van fysiologische functie en structurele veranderingen in meerdere organen. Gelijktijdig voorkomende comorbiditeiten worden vaak waargenomen bij patiënten met dementie of de ziekte van Parkinson, en bewijs uit epidemiologische studies heeft reeds de associatie aangetoond tussen systemische disfunctie en een hoger risico op het ontwikkelen van dementie. De rol van andere onderbelichte systemische functiestoornissen bij deze hersenaandoeningen is echter nog niet onderzocht. Een beter begrip van de relatie tussen systemische disfunctie, cognitie en hersenafwijkingen zou ons in staat stellen om een multi-systeem strategie te ontwikkelen voor de preventie van neurodegeneratie. Het overkoepelende doel van deze scriptie is om een beter begrip te krijgen van de relatie tussen systemische disfunctie en neurodegeneratieve aandoeningen, met name dementie en de ziekte van Parkinson, bij personen van middelbare leeftijd en ouder.

In **Hoofdstuk 2** heb ik onderzocht of een beperking van de longfunctie leidt tot verhoogde neurodegeneratie met behulp van ademhalingsonderzoeken en een klinische diagnose van chronische longziekte. In **Hoofdstuk 2.1** heb ik gerapporteerd dat een afname van de longfunctie samenhangt met een hoger risico op dementie. In **Hoofdstuk 2.2** heb ik onderzocht of een beperking van de longfunctie verband houdt met cognitieve prestaties en de aanwezigheid van vasculaire hersenlaesies. Ik heb aangetoond dat mensen met ernstigere COPD of een beperkte longfunctie slechtere algemene cognitieve prestaties hadden en een grotere prevalentie van lacunaire infarcten.

In **Hoofdstuk 3** heb ik gekeken naar de relatie tussen de prevalentie van neurodegeneratieve aandoeningen en leververvetting (FLD). Ik heb me in **Hoofdstuk 3.1** specifiek gericht op niet-

alcoholische leververvetting (NAFLD) en ontdekte dat noch NAFLD, noch fibrose in verband werd gebracht met een verhoogd risico op dementie. Daarnaast werd er geen verband gevonden tussen NAFLD en slechtere cognitieve functie. In **Hoofdstuk 3.2** vond ik een niet-significante relatie tussen FLD en parkinsonisme of de ziekte van Parkinson. Ik heb zowel abdominale echografie als de Fatty Liver Index gebruikt om leversteatose te diagnosticeren, en vond een negatieve associatie tussen leververvetting en neurodegeneratieve aandoeningen.

In **Hoofdstuk 4** heb ik onderzocht hoe cardiale biomarkers van invloed zijn op veranderingen in cognitie en structurele hersenveranderingen, evenals de incidentie van parkinsonisme en de ziekte van Parkinson. In **Hoofdstuk 4.1** heb ik longitudinale gegevens gebruikt om het effect van NT-proBNP op het verloop van cognitie en markers van hersenbeeldvorming met herhaalde metingen te onderzoeken. De resultaten toonden aan dat hogere baselinespiegels van NT-proBNP verband hielden met een snellere achteruitgang van de cognitie, maar niet met structurele veranderingen in de hersenen. In **Hoofdstuk 4.2** veranderde de focus van neurodegeneratie van het schatten van de trajecten van hersenmarkers naar de incidentie van neurodegeneratieve ziekten, specifiek parkinsonisme en de ziekte van Parkinson. Ik heb ontdekt dat hoge niveaus van cardiale biomarkers niet gerelateerd waren aan een verhoogd risico op de ziekte van Parkinson. Deze bevindingen kunnen bijdragen aan de kennis over de relatie tussen cardiale biomarkers en parkinsonisme en de ziekte van Parkinson, gezien het gebrek aan prospectieve studies over dit onderwerp.

In **Hoofdstuk 5** heb ik onderzocht hoe het risico op dementie werd beïnvloed door de botmineraaldichtheid in verschillende skeletgebieden bij oudere thuiswonende volwassenen. In

**Hoofdstuk 5.1** heb ik de analyses onderverdeeld in oplopende periodes van follow-up tijd en ontdekt dat deelnemers met een lage femurhals, een lage totale botmineraaldichtheid of een lage trabeculaire botkwaliteit een verhoogd risico hadden om dementie te ontwikkelen. Onze bevindingen die een verband leggen tussen botmineraaldichtheid en het risico op dementie, suggereren dat een lage botmineraaldichtheid mogelijk een gelijktijdig teken zou kunnen zijn tijdens de cognitieve achteruitgang die voorafgaat aan het optreden van dementie.

Tot slot, in **Hoofdstuk 6** besprak ik de belangrijkste bevindingen van deze scriptie, methodologische overwegingen, implicaties en toekomstig onderzoek. Wat betreft methodologische perspectieven, heb ik het kader van systemische functiestoornis besproken. Daarnaast besprak ik de mogelijkheid en impact van bias (confounding bias, information bias, en selection bias), evenals het probleem van omgekeerde causaliteit in de studies van deze scriptie. Ten slotte stelde ik de klinische implicaties voor en bood ik potentiële richtingen voor toekomstige studies aan.

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# Appendices

PhD Portfolio

| | |
|---|---|
| Name PhD student | Tian Xiao |
| Department | Epidemiology |
| Research school | Netherlands Institute for Health Sciences (NIHES) |
| PhD period | September 2019 – |
| Promotor | Prof. dr. M.A. Ikram |
| Co-promotor | Prof. dr. M.K. Ikram |

| **Training** | **Year** | **ECTS** |
|---|---|---|
| **1. Courses** | | |
| Repeated Measurements | 2021 | 1.7 |
| Principles of Genetic Epidemiology | 2021 | 0.7 |
| Joint models for longitudinal and Survival Data | 2021 | 0.7 |
| MGC OOA Genetic Engeerning in model organisms | 2021 | 1.8 |
| MGC Multiomics data integration in R | 2021 | 2.0 |
| Clinical Vascular Epidemiology | 2021 | 0.3 |
| Heart Failure Rsearch | 2021 | 0.3 |
| Epigenetic Regulation in Health and Disease | 2021 | 0.9 |
| Pulmonary Hypertension | 2021 | 0.3 |
| Kidney & Hypertension | 2021 | 0.3 |
| Advance in Clinical Epidemiology | 2022 | 0.7 |
| Microbiome Data Analysis in Population-based Studies | 2022 | 1.4 |
| Genome-wide Association Studies | 2022 | 1.4 |
| Basic and Translational oncology | 2022 | 1.8 |

| | | |
|---|---|---|
| SNPs and Human Diseases | 2022 | 2.0 |
| Scientific integrity | 2022 | 0.3 |
| Bayesian Statistics | 2022 | 0.6 |
| Biostatistics II | 2022 | 4.5 |
| Study design | 2022 | 4.0 |
| Selected topic in Epidemiology | 2022 | 3.0 |
| **2. Seminars** | | |
| Seminars at Epidemiology department | 2019-2023 | 4.0 |
| Seminar committee in Epidemiology | 2022-2023 | 1.0 |
| Journal Clubs | 2019-2022 | 2.0 |
| **3. Other activities** | | |
| Peer review for scientific journal (*Neurology*) | 2022 | 0.3 |

# List of Publications

**Xiao T**, Wijnant SRA, Licher S, et al. Lung Function Impairment and the Risk of Incident Dementia: The Rotterdam Study. J Alzheimers Dis 2021;82:621-630.

**Xiao T**, Wijnant SRA, van der Velpen I, et al. Lung function impairment in relation to cognition and vascular brain lesions: the Rotterdam Study. J Neurol 2022;269:4141-4153.

**Xiao T**, van Kleef LA, Ikram MK, de Knegt RJ, Ikram MA. Association of Nonalcoholic Fatty Liver Disease and Fibrosis With Incident Dementia and Cognition: The Rotterdam Study. Neurology 2022;99:e565-e573.

van Kleef LA, **Xiao T**, Ikram MA, Ikram MK, de Knegt RJ. Sex-stratified associations between fatty liver disease and Parkinson's disease: The Rotterdam study. Parkinsonism Relat Disord 2023;106:105233.

**Xiao T**, van der Velpen IF, Niessen WJ, et al. NT-proBNP and changes in cognition and global brain structure: The Rotterdam Study. Eur J Neurol 2023;30:2230-2239.

**Xiao T**, Ghatan S, Mooldijk SS, et al. Association of Bone Mineral Density and Dementia: The Rotterdam Study. Neurology 2023;100:e2125-e2133.

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## About the author

Tian Xiao was born on October 9th, 1992 in Jiangxi, China. He grew up in a hot and humid city surrounded by spicy foods and cuisines and studied medicine at the Gannan Medical University. During the last year of his studies, he was doing a rotation between diverse departments in a hospital and the local CDC, where his passion and desire for research started flaming up. For this reason, after obtaining her medical bachelor's degree in 2015, he passed the master's entrance examination at Fudan University and moved to Shanghai, China. There, he focused on COPD-related research and participated in studies in collaboration with other universities under the framework of the Sustainable Development Goal. In 2018, he obtained his Master's Degree in Epidemiology and later spent one year working at the CDC. At the same time, he felt puzzled about his life career and decided to make a change by developing himself in academia, which was believed to bring him an impulse of thrill and challenge. Between 2018 and 2019, he attended multiple job interviews for PhD programs and finally made up his mind to study brain health and cognition. For this reason, in 2019 she moved to Rotterdam and started her PhD training at the Epidemiology Department of Erasmus MC under the supervision of M. Arfan Ikram and M. Kamran Ikram, pursuing knowledge in the field of dementia and Parkinson's disease. During this time he was immersed in the etiology of neurodegeneration, collaborating with outstanding and truly kind researchers and colleagues within and outside of Erasmus MC. After graduation, he will perform postdoc research in Sweden with the expectation of transforming his research outcome into clinics.