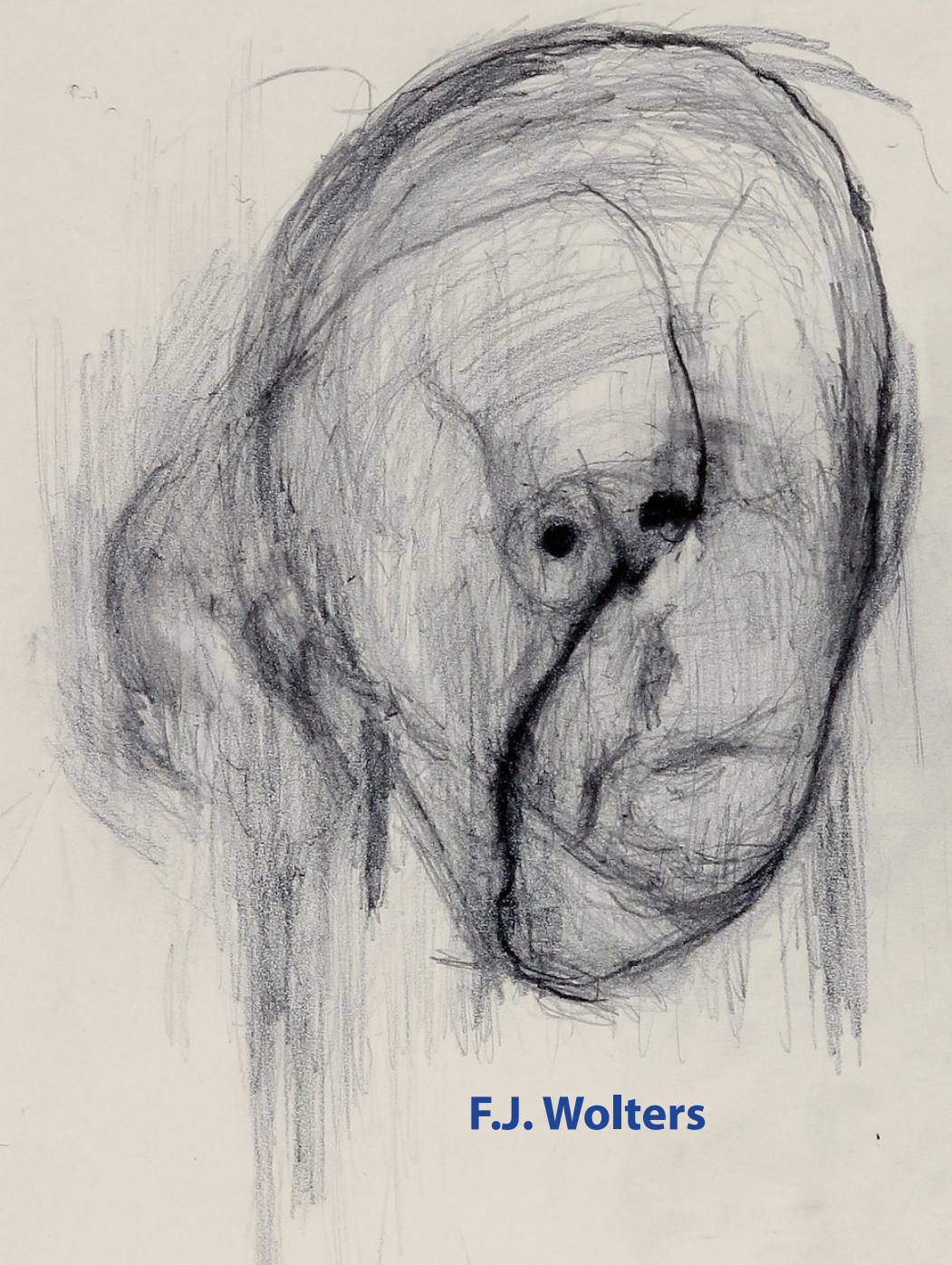
On the Origin *of* Dementia

A Population Perspective on Risk and Aetiology



F.J. Wolters

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On the Origin of Dementia
A Population Perspective on Risk and Aetiology

Over de Oorsprong van Dementie
Risico en Etiologie in Populatieperspectief

Proefschrift

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

Prof. dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

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Paranimfen	V.A. Kuiper S. Licher

To my parents

Forsan et haec olim meminisse iuvabit

– Virgil, *Anaeid* Book I

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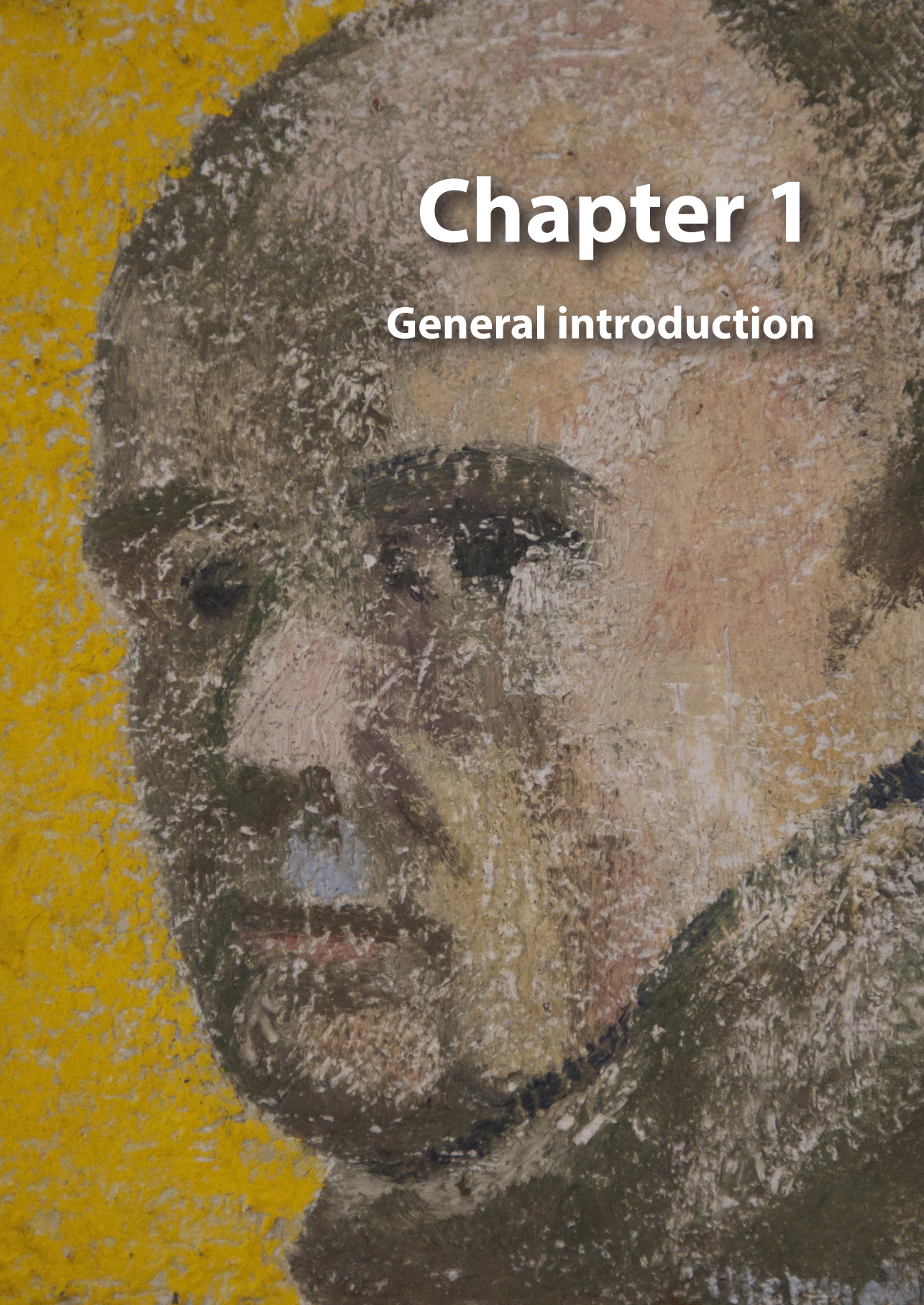
PROLOGUE

The first time I encountered the work of William Utermohlen was during a presentation at Brasenose College in Oxford, where one of the research fellows spoke about Alzheimer's disease, and highlighted a case from several years before at Queen Square, London. It was the case of an artist with great skill and a flourishing career who, after being diagnosed with Alzheimer's disease, decided to create a portrait of his own demise.

William Utermohlen (Philadelphia, PA, 1933) studied art at the Pennsylvania Academy of Fine Arts, and at the Ruskin School of Art in Oxford (UK), before settling in London in 1962. There he experienced his breakthrough as an artist, with notable exhibitions at the prestigious Marlborough gallery, and for decades to come Utermohlen would entice art observers with numerous portraits, still lives, and drawings. Yet, in the late 1980s something happens. Colour and composition start to change, perception of objects and people shifts, as the artist's work seems to enter a new thematic cycle. But in hindsight, these changes are no mere artistic evolution. As eloquently described by Dr. Patrice Polini in an analysis of Utermohlen's work from 1989 to 1991 (themed *Conversation Pieces*): "The artist excludes himself from the circles of talking figures, and when he does show himself, places his figure in a separate world: sleeping and dreaming (*Bed*), or communing with mute animals (*Snow*)." They are the premonitions of a gradually progressing disease.

In the following years, Utermohlen's style changes dramatically. Lines turn more abstract and colours darken, while anatomic positioning deteriorates. What for the patient attending a memory clinic is captured in a flawed double pentagon or the drawing of a clock is for the artist the gradual decline in his abilities on canvas. When Utermohlen eventually is diagnosed with Alzheimer's disease in 1995, this is the confirmation of process that had started many years before. It renders his series of self-portraits, as depicted on the cover of this edition, not only a unique collection of art, but also a precious medical document exemplifying the long-term change in ability and personality that precedes a diagnosis of dementia. From the perspective of a doctor and medical researcher, it implies that we need to focus on these first, very early changes, or perhaps even subclinical brain changes years prior to that, if we are to turn the tide of this disease.

Because of its powerful message, the story of William Utermohlen has been told many times, from documentaries like *L'oeil de Verre* (2009) to exhibitions by the *Wellcome trust* and publication in *The Lancet*. By visualising the inescapable deterioration in his series of self-portraits, Utermohlen has left us an urgent reminder that the development of preventive strategies against dementia deserves our utmost dedication.



Chapter 1

General introduction

GENERAL INTRODUCTION

Across the animal kingdom, the ability to acquire, process, and retrieve information allows to adapt to the environment, and for selected organisms the environment to their needs. Whether of our own making, or due to inevitable hazards of inhabiting this planet, the environment has always had a huge impact on the state of our brain, our mind, and our cognitive ability. Eighty-six billion neurons,[1] surrounded by an equal number of glial cells,[2] shape an interconnected network in our brain, so refined that it requires decades of environmental exposure, along with genetic susceptibility, to make it falter to the level of our awareness. But once it does, the consequences are atrocious. From subtle word finding difficulties to lost perception of time; from forgetfulness for a dentist appointment to a failure to recognise even those closest at heart.

At present, 48 million people worldwide are living with dementia, of whom the majority with Alzheimer's disease as its most common subtype. Due to ageing of the population, this number is predicted to double by 2040 (Figure 1). The immense burden of the disease not only falls upon the many patients, but is shared by countless caregivers, and a wider societal cost surpassing the $1 trillion mark in 2018. In the Netherlands, 1.5% of the population – 250,000 people – live with dementia, which despite strenuous efforts of roughly 300,000 caregivers, takes up about 7% (€5 billion) of the entire health care budget. The projections of rapid increases in the socio-economic burden of disease have led to widespread calls for prioritising dementia on the health agenda, with focus on prevention as the key to curbing this epidemic.[3-5] However, despite the overwhelming concern for global health, dementia remains understudied in terms of prevention at the population level,[6,7] and underfunded compared to other common high-burden conditions such as cancer and heart disease.[8] Recent years have seen a surge in investment in dementia research, but compared to other major common diseases, there is a substantial lag to overcome (Figure 2).

Most dementia research to date has focused on single pathophysiological mechanisms at the individual level. This has provided insights in specific biological pathways, but has been insufficient to provide an understanding of the full spectrum of dementia in the population. Indeed, the successive failure of various trials investigating potential disease-modifying treatments[9,10] suggests that the paradigm of a single target mechanism does not work well outside of the controlled laboratory and clinical environment. This multifactorial nature of dementia commonly emerges from population-based studies that have pinpointed various, mostly cardiovascular determinants of dementia in the general population. Together, modifiable risk factors like mid-life obesity, hypertension, and smoking account for roundabout 30% of dementia incidence,[4,5] but yet again, the underlying mechanisms by

which these risk factors lead to neurodegeneration remain elusive. The aim of this dissertation is to explore specific areas that I deem of aetiological importance to dementia, without losing sight of the full spectrum of the disease. After providing a bird's eye perspective of the occurrence of disease in **Chapter 2**, I shall therefore zoom in on cerebral haemodynamic mechanisms in **Chapter 3**, the interplay between dementia and cardiovascular disease in **Chapter 4**, and the role of the apolipoprotein E gene (*APOE*) in dementia and wider health outcomes in **Chapter 5**. As may become clear from the further presentation of these topics below, the thread by which this thesis is tied together is the aforementioned importance of prevention. This applies to clinical dementia, as well as the slowing of cognitive decline in innumerable spouses, parents, and otherwise engaged elderly who are prone to cognitive impairment that may not qualify as dementia, but certainly suffices to interfere with everyday undertakings, joy and quality of life, and mutual understanding with loved ones. For these reasons, throughout this dissertation my focus will be on dementia almost as much as it is on this subclinical decline in cognitive ability. In order to do so, the work presented in this thesis draws exclusively from population-based cohort studies, notably the Rotterdam Study, which I will therefore introduce in more detail.

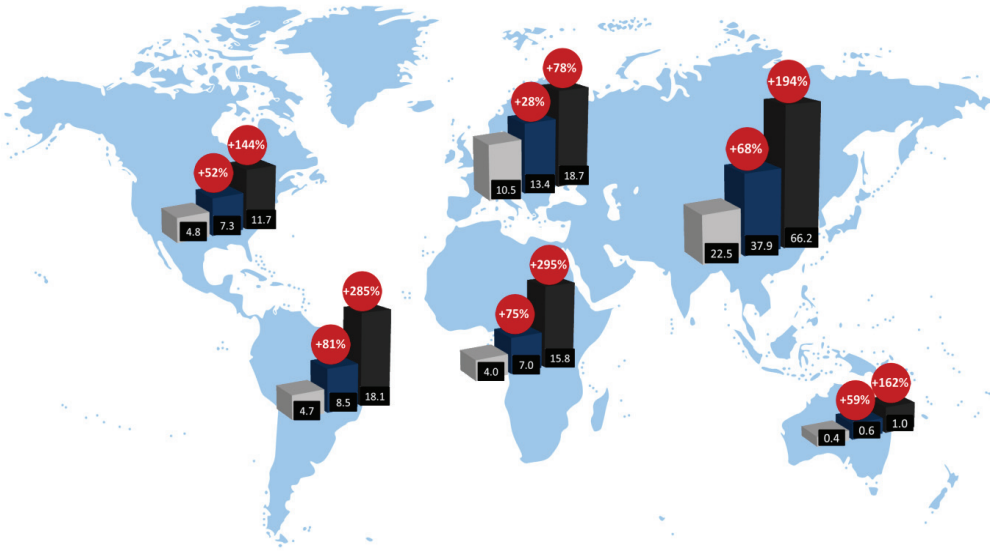


Figure 1. The number of people living with dementia in millions (black box) per geographic area in 2015 (light grey), with projections for 2030 (dark blue) and 2050 (dark grey). Corresponding percentages increase compared to 2015 are depicted in the red labels. Data source: World Alzheimer Report, 2015.

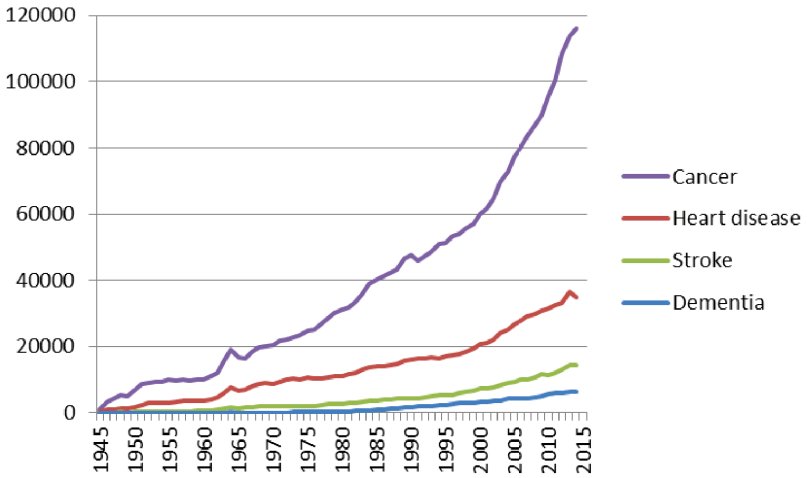


Figure 2. The number of scientific publications per year for different areas of research. Numbers are obtained from the PubMed library.

The Rotterdam Study, locally known as Erasmus Rotterdam Gezondheid Onderzoek (ERGO), was established in 1989 to investigate the occurrence and determinants of common diseases in the elderly.[11] Designed as a geographically defined population-based cohort, the study keeps track of over 15,000 inhabitants, aged 40 years and older, of the Ommoord suburb of Rotterdam. Through four-yearly research centre visits, and permission to continuously monitor their health status through general practitioner records, these loyal and dedicated people have now allowed careful study of neurological disease and heart disease, in addition to a variety of other organ systems for nearly three decades (Figure 3).[12] Although the Rotterdam Study at time of its inception was certainly not the first of its kind, it was one of the few studies with a focus on neurodegenerative disease. The relevance of this is quickly appreciated when viewing the scarcity of dementia research at the time, compared to for example heart disease and cancer (Figure 2). The 28 years of follow-up that have since been amassed render the Rotterdam Study a valuable tool to map the burden of disease, and unravel the long pre-clinical course of dementia.[13] Of note, the Rotterdam Study has been approved by the medical ethics committee according to the Population Screening Act Rotterdam Study, as executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all its participants.

Data from the Rotterdam Study are yielded first in **Chapter 2** to provide estimates of the occurrence and burden of dementia based on 27 years of observations in the Dutch population, which may serve public awareness and informed decision-making by policy makers alike. The healthcare adaptations needed to prepare for the increasing burden of disease thereby not only depend on the risk of developing dementia, but equally on the

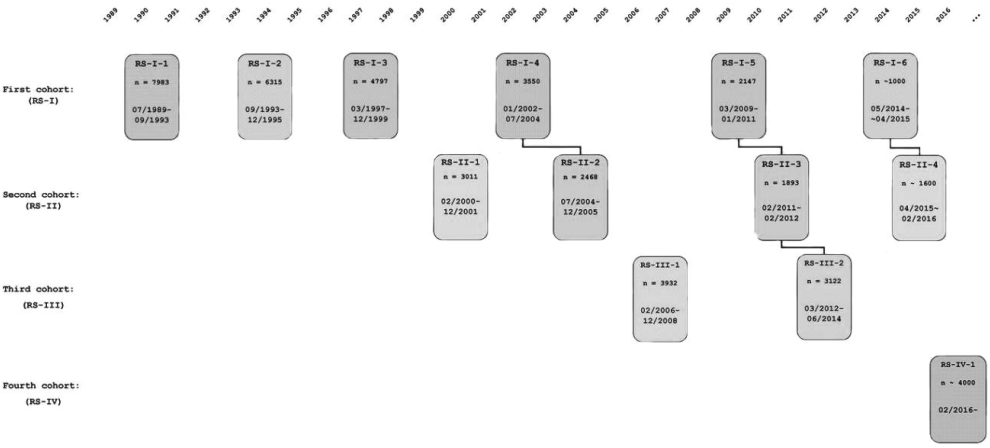


Figure 3. Design of the Rotterdam Study, showing all examination cycles to date of the four inclusion waves.

(expected) number of years lived with disease in the context of overall life expectancy. For this reason, in **Chapter 2**, I describe both lifetime risks of dementia, in the context of other common brain diseases, and the expected number of years lived with dementia. Although these estimates originate from careful observations, it is important to note that projections for the future may vary with changes in disease incidence. Such time trends are therefore investigated in **Chapter 2,** by clustering observations from five European countries and the United States. I conclude this chapter by providing a glimpse of the preventive potential for dementia by interventions at the population level.

In **Chapter 3** of this thesis I shall investigate essentially one aetiological question: is disruption of blood supply to the brain an important factor in the pathogenesis of dementia? It has long been acknowledged that abrupt, severe hypoxia, leading to ischaemic stroke, greatly increases one's chances of developing dementia.[14] But most of the exposure to cerebral blood flow reduction, and potentially hypoxia, is transient and may go by unnoticed. The brain is a highly vascularised organ, receiving 15% of cardiac output and accounting for about 20% of the body's total oxygen consumption despite comprising less than 3% of body weight.[15] Their large metabolic demand renders neurons sensitive to disruption in nutrient supply, which is why several regulatory mechanisms are in place to maintain continuous cerebral perfusion. Despite this delicate equilibrium, however, the consequences of transient episodes or chronic stages of reduced cerebral perfusion on neurodegeneration and cognitive decline remain largely undetermined. These are complicated by the fact that the loss of neuronal cells reduces metabolic demand, and consequently blood supply, long before the brain falters to the level of clinical dementia. Long-term observations are therefore needed, founded firmly upon the principles of cerebral haemodynamic physiology.

In physiological conditions, cerebral blood flow (CBF) is proportional to the cerebral metabolic rate, and in resting state equals about 50-60 mL per 100mL of brain tissue per minute. Haemodynamically, CBF is a resultant of the cerebral perfusion pressure (CPP) and the cerebrovascular resistance (CVR) (as expressed by Ohm's law: $CBF = \frac{CPP}{CVR}$).[16] The CPP is the pressure gradient that drives cerebral blood flow, depending on mean arterial pressure (MAP) and intracranial pressure (ICP) ($CPP = \frac{MAP}{ICP}$). The arterial pressure component is determined by the cardiac output, systemic vascular resistance, and central venous pressure (CVP) ($MAP = CO * SVR + CV$).[17] Compared to regular MAP of around 95 mmHg, ICP is relatively low under physiological circumstances (7-15 mmHg in supine position). Nevertheless, it modulates flow by constituting the interstitial pressure that limits capillary filtration from the intracranial capillaries, and to a lesser extent through compression of the cerebral vessels. Regulation of CVR, however, is mostly under metabolic control (through hypercapnia and to a lesser extent hypoxia), supported by neural regulation (i.e. via release of vasoactive neurotransmitters), and myogenic control (i.e. changes in transmural pressure).[18] As the ICP cannot be reliably determined non-invasively, it has often been attempted to estimate the CVR otherwise. Notable examples are the pulsatility index ($PI = \frac{Vsystole - Vdiastole}{Vmean}$) and the (highly correlated) restivity index ($RI = \frac{Vsystole - Vdiastole}{Vsystole}$), which were coined by respectively Gosling and Pourcelot in the 1970s using transcranial Doppler.[19,20] However, despite the usefulness of Gosling's index in assessing intracranial artery pulsatility, it may not capture well the CVR.[21]

Cerebral perfusion pressure is held fairly constant due to various autoregulatory mechanisms that safeguard blood supply to the brain. These mechanisms rely both on autonomic nervous system function and cerebrovascular reactivity. The former includes chronotropic and inotropic effects on the heart and arterial and venous constriction due to effects on vascular smooth muscle cells, and influence variation in resting conditions as well as response to for example an orthostatic challenge. Within the brain, neurons, glia, and cerebral blood vessels function as an integrated unit to adjust blood supply to changes in metabolic demand, a process known as neurovascular coupling. This local vasoreactivity acts predominantly through changes in cerebrovascular resistance, and maintains cerebral blood flow as long as arterial pressure is within the range of about 60-150 mmHg. Below a certain perfusion pressure, however, the local autoregulatory mechanism falters, and cerebral blood flow starts to decline (Figure 4). To maintain neuronal metabolism, oxygen extraction then increases, which puts forward arterial oxygen content (i.e. haemoglobin concentrations and oxygen saturation) as a factor of importance in the development (and prevention) of neuronal hypoxia and ischaemia with drops in perfusion pressure.

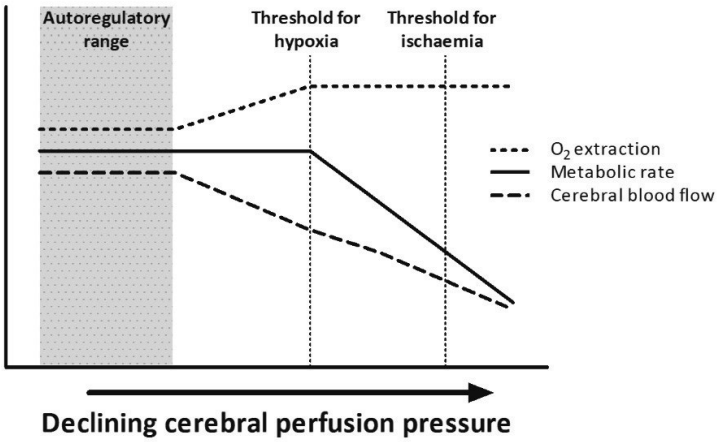


Figure 4. Schematic overview of changes in metabolism with declining cerebral perfusion pressure. Protein synthesis gradually reduces from about 50% of its capacity with cerebral blood flow of 55mL/100mL/min to complete suppression at 35mL/100mL/min. With further lowering of perfusion electroencephalographic amplitudes start to decrease, and at about 15-20mL/100mL/min ATP breakdown is soon followed by anoxic depolarisation of cell membranes and disappearance of evoked potentials.[22]

With these haemodynamic principles in mind, I investigate in **Chapter 3** the long-term consequences of low cerebral perfusion, and of its regulatory mechanisms on the risk of dementia. This chapter concludes by assessing the effect of carotid artery stenosis on imaging markers of neurodegeneration. **Chapter 4** subsequently focuses on the link between heart and brain, and probes potential haemodynamic or thromboembolic complications of heart disease on cognitive health, while exploring hallmarks of Alzheimer's disease in light of systemic vascular disease.

In **Chapter 5**, I shall direct attention to what is arguably the most notorious of risk factors for Alzheimer's disease: The Apolipoprotein E (*APOE*) gene. Rarely in the realm of medicine does one encounter such an important common genetic risk factor. Since its implication in Alzheimer's disease in 1993,[23] much has been said and written about the role of *APOE* in dementia.[24] However, the contemporary identification of *APP* and *PSEN1/PSEN2* as autosomal dominant Alzheimer genes has undoubtedly framed much of the attention for *APOE* in the context of the amyloid hypothesis. This has in my view left various other systemic effects of *APOE*, notably involving lipid metabolism and atherosclerosis,[25,26] underappreciated, and the consequences of *APOE* on disease outside the central nervous system under-investigated. Moreover, the vast majority of research has focused on the high-risk ε4 allele, with little attention for the apparent protective effects of the ε2 allele.[27] This is partly driven by the lower allele frequency, approximating 8% for ε2 versus 78% for ε3 and

14% for ε4,[28] necessitating sizeable study populations to disentangle effects of the ε2 from that of the ε3 allele.

Apart from the aetiological insights that *APOE* offers, its substantial risk estimates render it a suitable candidate for risk prediction of dementia in the community.[28] Reliable risk stratification is important for clinical decision-making, and has gained considerable interest in the selection of individuals for participation in clinical trials. However, available risk prediction models display poor calibration and show no better discriminative accuracy than prediction based on age alone.[29] Yet, these models are chiefly based on demographics and environmental risk factors. Heritability of Alzheimer's disease has been estimated as high as 60-70% on the basis of twin studies,[30] and although potentially still mediated by environmental factors, the high heritability suggests that genetic factors may be used to distinguish individuals at low and high risk of dementia in the population. Indeed, the hitherto identified common genetic risk variants seem to hold some promise for risk stratification, but validation of these results in prospective population-based studies is mandatory before these could be applied in clinical setting. Moreover, given that much of the heritability of Alzheimer's disease remains yet unexplained, it would be unwise to omit a classic family history of dementia from patient interview and investigation, and possibly incorporation in prediction rules. In **Chapter 5** I shall therefore investigate the effect of *APOE,* and in particular the ε2 allele, on lipid fractions and mortality risk in the population, and yield genetic determinants of dementia, including *APOE* along with other genetic variants and family history, for predictive purposes in the community.

I aspire that this thesis will ultimately provide a few answers, and above all a clearer picture of the questions lying before us. To wander a short distance down that road, I shall reflect on the content of this thesis and share my views on its implications in **Chapter 6**. Take these contemplations as an invitation for further debate, for the end of any journey is just the beginning of another, and it is beyond doubt that scientific debate will be much needed if we are to achieve the full potential for prevention of dementia.

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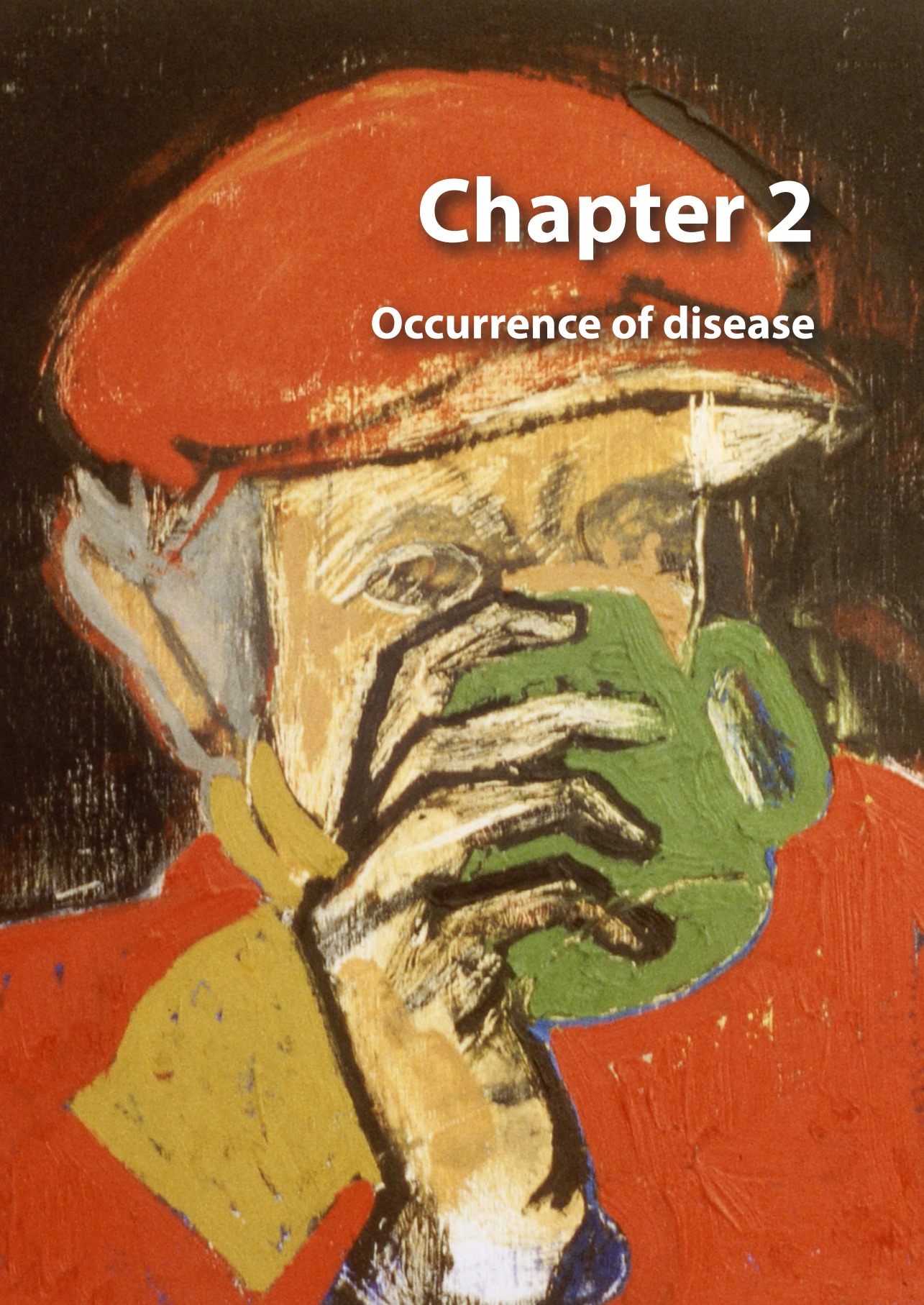
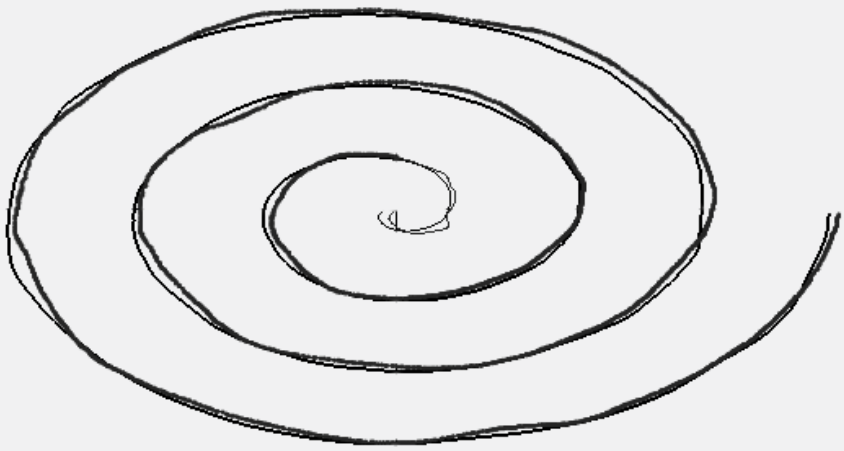


Chapter 2

Occurrence of disease

Chapter 2.1

Life-expectancy



ABSTRACT

Reliable estimates of life expectancy and associated disability in the population are important for shaping health care policy, but a limited number of studies provide the prospective life-course data required for reliable estimates regarding dementia. Yielding data from 10,348 persons (median age 67 years, 60% women) from the population-based Rotterdam Study, who were followed from 1990-2015 for the occurrence of dementia and death, we created multi-state lifetables, based on prevalence, incidence rates, and hazard ratios for three transitions. We also assessed the effect of postponing disease onset on life expectancy with and without dementia. During 120,673 person-years, 1,666 (17.0%) persons developed dementia, and 6,150 (59.4%) died. Overall life-expectancy of women ranged from 18.0 years (95% confidence interval: 17.8-18.2) at age 65 to 2.3 years (2.2-2.3) at age 95. Of total life-expectancy at age 65, 5.7% (1.0 year, 1.0-1.1) was lived with dementia, increasing with age to 42.1% (1.0 year, 0.9-1.0) of life expectancy at age 95. For men, overall life-expectancy ranged from 15.6 years (15.4-15.9) at age 65 to 1.8 years (1.7-1.8) at age 95, of which 3.7% (0.6 year, 0.5-0.6) and 35.3% (0.6 year, 0.5-0.7) was lived with dementia, respectively. Postponing the onset of dementia by 1 to 3 years resulted in 25 to 57% reductions in years lived with dementia. Having attained primary education only was associated with higher mortality and dementia rates, resulting in higher shares of remaining years lived with dementia. Survival after dementia diagnosis ranged from 6.7 (95% confidence interval: 5.3-8.1) years when diagnosed before age 70, to 2.6 years (2.3-2.9) after age 90, longer for women than men, but unaffected by educational attainment. In conclusion, dementia places a large burden on individuals and society in terms of healthy life-years lost, but this is potentially highly amendable by preventive interventions at the population level.

INTRODUCTION

At present, 48 million people worldwide live with dementia, and due to a rapidly ageing populations this number is expected to rise to 131 million by 2050.[1] As effective preventive and curative interventions are lacking, the already enormous burden on patients, their caregivers, and health care systems will increase further, and monetary costs of dementia will reach a yearly one trillion USD as soon as 2018.[1] This foresight has led to a widespread call to render dementia a public health priority,[2] yet standards of care for patients remain often below par,[3,4] and public health intervention lags behind to timely facilitate improvement.[5] To better allow health care policy to adapt to these challenges, understanding the disease burden in terms of years lived with disability, and healthy life years lost is paramount.[6]

Various studies have assessed the prognosis of dementia in the general population, in terms of survival after diagnosis.[7-10] Although invaluable to inform clinicians, patients, and their relatives, the application of these data to public health interventions requires to see them in light of population structure, underlying dementia risks, and life expectancy without dementia. Few studies, however, provide the comprehensive life course data required to fulfill this objective, with careful ascertainment of dementia and mortality over prolonged follow-up in the community. Consequently, life expectancy without and with dementia has been less well studied than prognosis after disease onset. The few published studies date back to the 1970s and '80s,[11-13] were modelled using only prevalence data of dementia[13,14] or follow-up data limited to three years,[12] or rely in part on simulations rather than empirical data for calculation of life expectancy.[15] Moreover, these estimates may vary by population characteristics that influence dementia risk and survival, such as sex, educational attainment, and the apolipoprotein E (*APOE*) genotype as the most important common genetic risk factor for dementia. As improvements in education are put forward in preventive strategies against dementia, and various clinical trials against Alzheimer's disease now focus on high-risk *APOE*-ε4 carriers, insight in the effect of these characteristics in the population is elementary to tailor these interventions and map changes in the burden of disease.

We therefore aimed to investigate life expectancy, segregated by years lived with and without dementia, using multi-state life tables with data from the longstanding population-based Rotterdam Study.

METHODS

Study population

This study is embedded within the Rotterdam Study, a large population-based cohort study in the Netherlands, details of which have been described previously.[16] Briefly, the original study population in 1990 consisted of 7,983 participants aged ≥55 years from the Ommoord area, a suburb of Rotterdam. In 2000, the cohort was expanded with 3,011 persons who had reached age 55, or moved into the study area. Participants undergo extensive follow-up examinations at a dedicated research center every 4 years. The current study includes all 10,520 participants who underwent sufficient cognitive screening to determine dementia status at study entry.

Assessment of mortality

Information on vital status was obtained through automated linkage of general practitioner files with the study database, as well as a bimonthly check of municipal records.[16] Follow-up for mortality until 1st January 2015 was complete.

Dementia screening and surveillance

Participants were screened for dementia at baseline and subsequent center visits with the Mini-Mental State Examination (MMSE) and the Geriatric Mental State Schedule (GMS) organic level.[17] Those with an MMSE score <26 or GMS score >0 underwent further investigation and informant interview, including the Cambridge Examination for Mental Disorders of the Elderly. In addition, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study center with medical records from general practitioners and the regional institute for outpatient mental health care. A consensus panel headed by a consultant neurologist established the final diagnosis according to standard criteria for dementia (DSM-III-R). Follow-up for dementia until 1st January 2015 was virtually complete (97.8% of potential person-years). Within this period, participants were censored at date of dementia diagnosis, death, loss to follow-up, or 1st January 2015, whichever came first.

Other measurements

Educational attainment was ascertained at study entry by interview, and classified into primary education only, further education (i.e. lower or intermediate vocational, or general secondary education) and higher education (i.e. higher vocational or university education). We assessed marital status, history of smoking (i.e. current, former, never), and use of antihypertensive or lipid-lowering medication at baseline by interview. Systolic and diastolic blood pressures were measured with a random-zero sphygmomanometer; the mean of two

readings was used for analysis. Blood samples were obtained at baseline to determine serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and glucose. Diabetes was defined as the use of blood glucose-lowering medication at baseline, a fasting serum glucose level ≥7.0 mmol/L (126 mg/dL), or a non-fasting serum glucose level ≥11.1 mmol/L (200 mg/dL). Body mass index was computed from measurements of height and weight (kg/m^2). Information on the prevalence of cancer, chronic obstructive pulmonary disease, and cardiovascular disease (i.e. stroke, myocardial infarction, heart failure and atrial fibrillation) was obtained by interview and inspection of medical records. *APOE* genotype was determined by polymerase chain reaction on coded DNA samples in the original cohort, and by bi-allelic Taqman assays (rs7412 and rs429358) for the expansion cohort. In 258 participants with missing *APOE* status from this blood sampling, genotype was determined by genetic imputation (Illumina 610K and 660K chip; imputation with Haplotype Reference Consortium (HRC) reference panel (v1.0) with Minimac3). Overall, *APOE* genotype was determined in 91.4% of participants, and classified as *APOE*-ε4 carrier (≥1 ε4 allele) or non-carrier.

Analysis

Participants were included in the analysis from the moment they reached age 65 onwards. Of 10,520 eligible participants, 144 (1.4%) died prior to age 65, and 28 (0.3%) were lost to follow-up for dementia prior to reaching this age, leaving 10,348 participants for the analyses. For participants with delayed entry (i.e. aged <65 years at study entry) covariate data of the closest study visit were used. Missing data for covariates were imputed using the mean of fivefold multiple imputations. This applied to missing data for education (3.4%), *APOE* genotype (8.5%), marital status (3.8%), body mass index (12.1%), total cholesterol (8.4%), HDL cholesterol (8.8%), statins (0.6%), systolic and diastolic blood pressure (7.6%), blood pressure lowering medication (0.8%), smoking (4.6%), and diabetes (12.1%). For the stratified analyses, imputed data for education and *APOE* were not included.

We created multistate lifetables to calculate life expectancy, defining three health states, namely non-demented, demented, and deceased.[18-20] Unidirectional transition between these states was possible from non-demented to demented, from non-demented to deceased, and from demented to deceased. We obtained the age-specific transition rates for each transition. Dementia prevalence was then determined per 10-year age groups (i.e. 65-74; 75-84; 85-94; ≥95). Next, we determined hazard ratios of incident dementia and death using Poisson regression with the Gompertz distribution (most suitable to model exponential increases with age), comparing 1) women to men, 2) individuals with further and higher education to primary education only, and 3) carriers of 1 or 2 *APOE*-ε4 alleles to non-carriers. This model was adjusted for age, birth year (to address potential cohort effects),

educational attainment (if applicable), *APOE* genotype (if applicable), marital status, smoking, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, systolic and diastolic blood pressure, blood pressure lowering medication, body mass index, diabetes, and prevalent cancer, chronic obstructive pulmonary disease, or cardiovascular disease. We stratified analyses by sex, and additionally determined differences in life expectancy with and without dementia across three levels of educational attainment, and separately for *APOE*-ε4 carriers and non-carriers. We then constructed the multistate lifetables using the overall transition rates, dementia prevalence, and adjusted hazard ratios. Sex-specific lifetables started at age 65 and closed at age 100. In addition, we calculated the percentage of remaining life years lived with dementia by dividing years lived with dementia by the life expectancy at each year of life. We used Monte Carlo simulation (parametric bootstrapping) with 10,000 runs to calculate the confidence intervals of (differences in) life expectancy estimates with @RISK software (Palisade Corporation, NY, USA). We repeated the sex-stratified analyses for hypothetical scenarios in which the onset of dementia was delayed by 1, 2, and 3 years, respectively, while keeping mortality constant, and determined the reduction in years lived with dementia compared to the empirical data.

Finally, proceeding to prognosis of individual patients with dementia, we determined survival after dementia diagnosis in all individuals who were diagnosed with dementia during follow-up until 1st January 2013, using the Kaplan-Meier estimator. For these individuals, additional mortality data were ascertained up till June 2017, and 1397/1526 (91.5%) participants with dementia had died within this follow-up period. We calculated the percentage of expected life years lost due to diagnosis of dementia, by dividing the median survival after diagnosis by the overall life expectancy at ages 65-69 and 85-89.

Analyses were done using IBM SPSS Statistics version 21.0 (IBM Corp, Armonk, NY, USA), Microsoft Excel 2010 (Microsoft Corp, Redmond, WA, USA), and Stata version 14.1 (StataCorp, College Station, TX, USA).

RESULTS

Table 1 shows the baseline characteristics of the study population. The median age at baseline was 67.3 years (range 65-106 years), and 59.6% were women.

Characteristics	Study population
Age at study entry, years (median, range)	67.3 (65-106)
Age at dementia diagnosis, years	83.4 (±6.6)
Women	6172 (59.6%)
Educational attainment	
Primary education	2118 (21.2%)
Further education	6814 (68.1%)
Higher education	1068 (10.7%)
APOE genotype	
ε4 non-carriers	6819 (72.0%)
ε4 carriers	2652 (28.0%)
Marital status	
Living with partner	6487 (65.2%)
Unmarried, widower, or living apart together	3468 (34.8%)
Smoking	
Never	3563 (36.1%)
Former	4381 (44.4%)
Current	1925 (19.5%)
Systolic blood pressure, mmHg	143 (±22)
Diastolic blood pressure, mmHg	76 (±12)
Antihypertensive medication	3670 (35.7%)
Body mass index, kg/m^2	26.8 (±4.0)
Diabetes	1002 (11.0%)
Serum total cholesterol, mmol/L	6.3 (±1.2)
Serum high-density lipoprotein cholesterol, mmol/L	1.4 (±0.4)
Lipid-lowering medication	862 (8.4%)
History of cardiovascular disease	1564 (15.1%)
History of cancer	410 (4.0%)
History op chronic obstructive pulmonary disease	515 (5.0%)

Table 1. **Population characteristics of the 10,348 participants.** Data are presented as means (±standard deviation) for continuous variables and absolute numbers with percentages for categorical variables, of the non-imputed data, unless stated otherwise. *APOE*=apolipoprotein E.

Risk of dementia and death

Of all 10,348 participants, 521 (5.0%) were diagnosed with dementia at baseline. During 120,673 person-years of follow-up (mean 11.7 years), 1,666 (17.0%) participants developed dementia, and 6,150 (59.4%) died. Women were at higher risk of dementia than men (hazard ratio (HR), 95% confidence interval: 1.19, 1.05-1.36), whilst they were at lower risk of death (for non-demented, HR 0.68, 0.64-0.73; and for demented, HR 0.76, 0.66-0.87). Table 2 shows the sex-stratified associations of educational attainment and *APOE* genotype with risk of dementia and mortality. Higher educational attainment was associated with a lower risk of dementia and death among non-demented individuals, and relative risk

estimates for dementia thereby exceeded those of mortality (Table 2). The presence of one or two *APOE*-ε4 alleles was associated with an increased risk of dementia, as well as a more modest increase in risk of death in non-demented, but not in demented individuals (Table 2).

Life expectancy and years lived without and with dementia

Life expectancy of women ranged from 18.0 years (95% confidence interval: 17.8-18.2) at age 65 to 2.3 years (2.2-2.3) at age 95. Of total life expectancy at age 65, 5.7% (1.0 year, 1.0-1.1) were lived with dementia, increasing with age to 42.1% (1.0 year, 0.9-1.0) of life expectancy at age 95 (Figure 1). For men, overall life expectancy ranged from 15.6 years (15.4-15.9) at age 65 to 1.8 years (1.7-1.8) at age 95, of which 3.7% (0.6 year, 0.5-0.6) and 35.3% (0.6 year, 0.5-0.7) were lived with dementia, respectively (Figure 1).

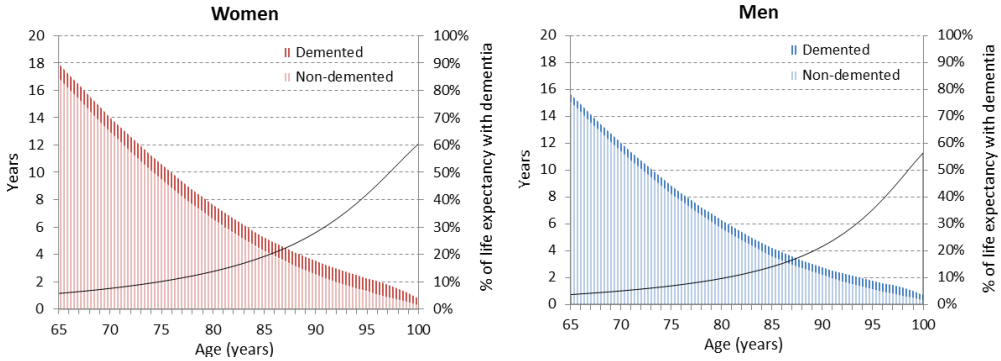


Figure 1. Total life expectancy with and without dementia. The life expectancy per year from the age of 65 years, for women (left) and men (right). Bars represent the total number of years lived (labelled on the left Y-axis), segregated by years without dementia (light color) and with dementia (dark color). The solid line reflects the percentage of remaining lifeyears lived with dementia (labelled on the right Y-axis).

In three different scenarios for preventive interventions against dementia, delaying the onset of dementia in the population by one, two, or three years resulted in reductions in the number of observed incident cases from 1666 to 1417, 1154, and 886, respectively. The number of life years lived with dementia thereby decreased with 25% in the one-year scenario, with 46% in the two-year scenario, and with 57% in the three-year scenario, broadly similar for women and men (Figure 2).

Effect of educational attainment and *APOE* genotype

Overall life expectancy differed across levels of educational attainment (Figure 3). Highly educated 65-year olds were expected to live on average a year and a half longer than those of similar age with primary education only (mean difference [95% CI] for women: 1.6 [0.6-2.5] years; and for men 1.2 [0.4-2.1] years). Due to reductions in both mortality and

dementia risk with higher education, educational attainment did not materially affect the number of years lived with dementia (for highly educated women -0.3 [-0.7;0.1] years, and for men 0.0 [-0.3;0.3] years). However, as individuals with only primary education on average lived shorter, the share of remaining life expectancy lived with dementia was substantially greater than in those with further or higher education, in particular for women (Figure 3).

APOE-ε4 carriership was associated with on average 2.4 (2.1-2.8) healthy life years lost in women, and 1.7 (1.2-2.1) years in men. This effect was due to a reduction in overall life expectancy at age 65 of 1.3 (1.0-1.7) years in women and 1.1 (0.7-1.6) years in men, along with an increased number of years lived with dementia (at age 65 in women: 1.1 [0.9-1.3]; and for men: 0.5 [0.4-0.7]). This resulted in a percentage share of remaining life expectancy lived with dementia of 71.3% at age 95 in female carriers compared to 38.8% at the same age in non-carriers (Figure 4). In men, these percentages were slightly lower at 64.4% and 31.9%, respectively (Figure 4).

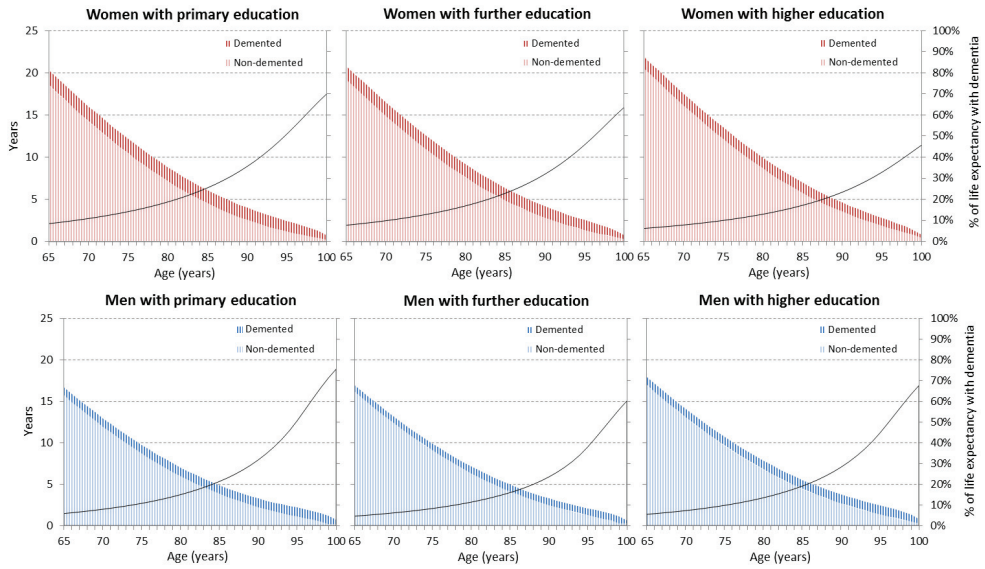


Figure 3. Life expectancy with and without dementia, stratified by educational attainment. The life expectancy per year from the age of 65 years, for women (top row) and men (bottom row), stratified from left to right by educational attainment. Bars represent the total number of years lived (labelled on the left Y-axis), segregated by years without dementia (light color) and with dementia (dark color). The solid line reflects the percentage of remaining lifeyears lived with dementia (labelled on the right Y-axis).

	Risk of dementia Hazard ratio (95% CI)		Mortality among non-demented Hazard ratio (95% CI)		Mortality among demented Hazard ratio (95% CI)	
	Women	**Men**	**Women**	**Men**	**Women**	**Men**
Educational attainment						
Primary	REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE
Further	0.86 (0.76-0.99)	0.82 (0.65-1.04)	0.94 (0.87-1.02)	0.95 (0.85-1.06)	0.97 (0.86-1.11)	1.13 (0.91-1.40)
Higher	0.67 (0.48-0.94)	0.78 (0.56-1.07)	0.79 (0.65-0.95)	0.85 (0.73-0.99)	1.03 (0.72-1.47)	0.85 (0.62-1.16)
***APOE* genotype**						
ε4 non-carriers	REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE
ε4 carriers	2.25 (1.99-2.55)	2.20 (1.84-2.64)	1.15 (1.05-1.24)	1.12 (1.02-1.23)	0.98 (0.87-1.11)	1.00 (0.84-1.21)

Table 2. Associations of educational attainment and *APOE* genotype with risk of dementia and death by sex. Models are adjusted for age at study entry, birthyear, educational attainment, *APOE* genotype, marital status, cardiovascular risk factors, and comorbidity. HR=hazard ratio; CI=confidence interval.

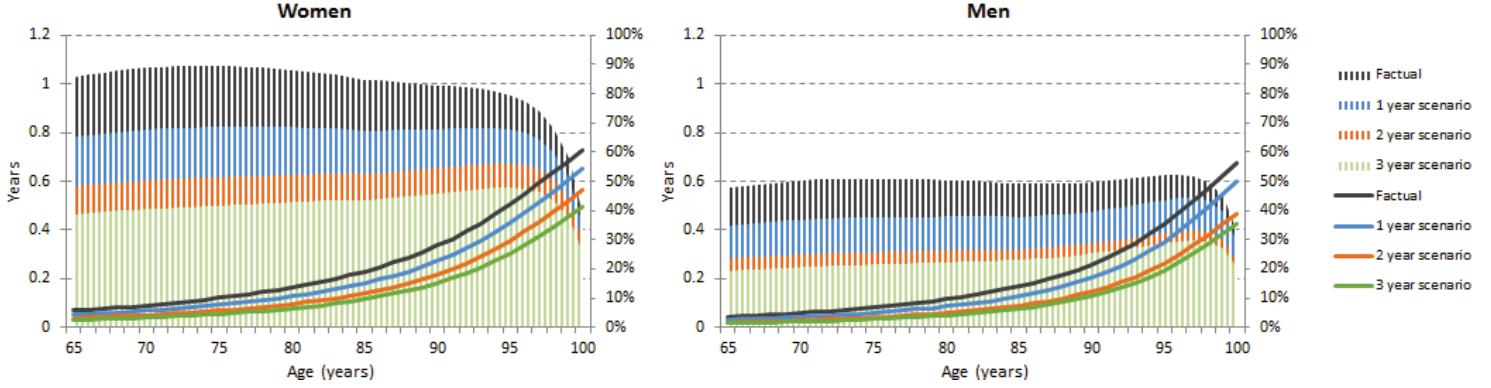


Figure 2. The effect of preventive intervention against dementia on the expected lifeyears lived with dementia, in absolute numbers and as a share of the total remaining life expectancy, for women (left) and men (right). Bars represent the total number of years lived with dementia (labelled on the left Y-axis), comparing the factual data (corresponding to Figure 1) with hypothetical scenarios in which the onset of dementia is delayed by 1, 2, and 3 years, respectively. For the same scenarios, the solid lines reflect the percentage of total remaining lifeyears that is expected to be lived with dementia (labelled on the right Y-axis).

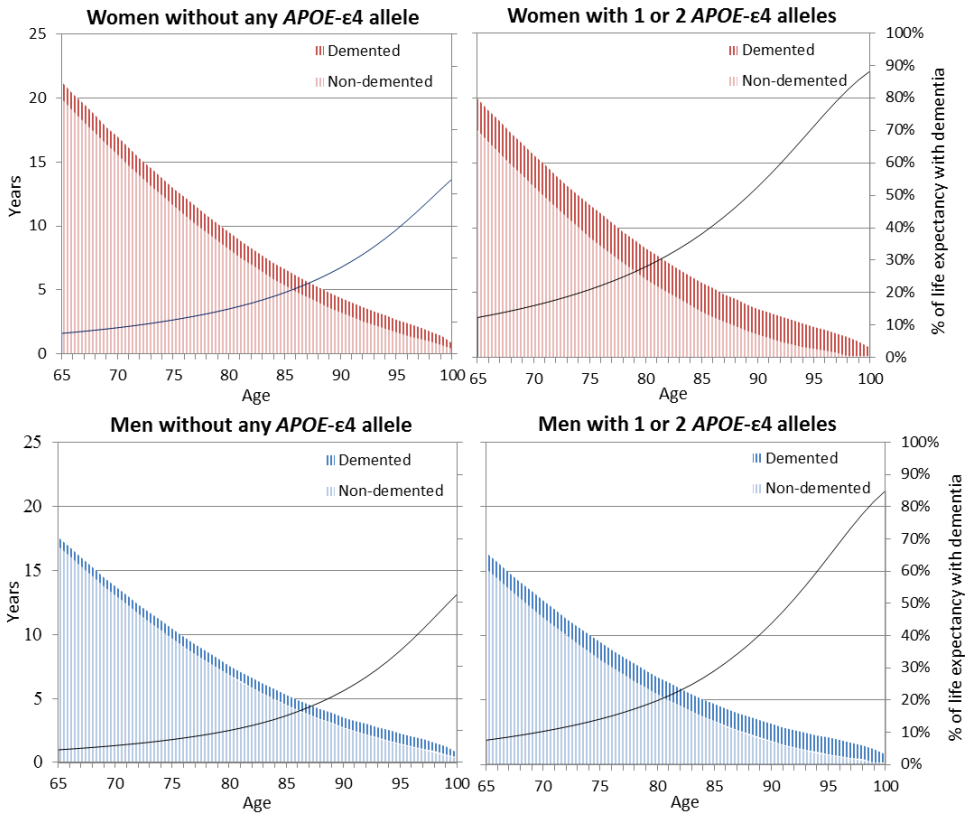


Figure 4. Life expectancy with and without dementia, stratified by *APOE* genotype. The life expectancy per year from the age of 65 years, for female ε4 non-carriers and carriers (top row) and male ε4 non-carriers and carriers (bottom row). Bars represent the total number of years lived (labelled on the left Y-axis), segregated by years without dementia (light color) and with dementia (dark color). The solid line reflects the percentage of remaining lifeyears lived with dementia (labelled on the right Y-axis).

Prognosis after dementia diagnosis

Median survival after diagnosis of dementia was 3.7 years (95% CI 3.5-3.9), but varied with sex and, in particular, with age at diagnosis (Table 3). In women, median survival ranged from 7.7 years when diagnosed before age 70, to 2.6 years with diagnosis after the age of 90. Among men, these numbers were 5.3 years and 2.4 years, respectively. A diagnosis of dementia was thus associated with a reduction in median life expectancy of close to 60% (53% for women and 63% for men) when diagnosed between 65-69 years, and by about 20% (24% for women and 21% for men) when diagnosed aged 85-89. Prognosis was not affected by educational attainment or *APOE*-ε4 carrier status.

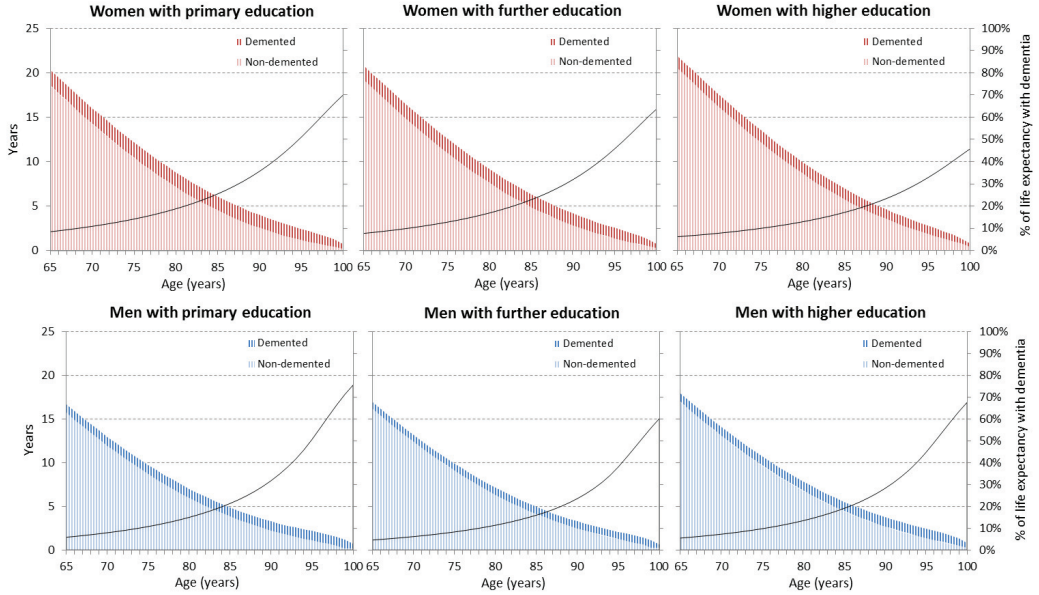


Figure 3. Life expectancy with and without dementia, stratified by educational attainment. The life expectancy per year from the age of 65 years, for women (top row) and men (bottom row), stratified from left to right by educational attainment. Bars represent the total number of years lived (labelled on the left Y-axis), segregated by years without dementia (light color) and with dementia (dark color). The solid line reflects the percentage of remaining lifeyears lived with dementia (labelled on the right Y-axis).

	Men		Women	
	N_t/N_{dem}	Median survival (95% CI)	N_t/N_{dem}	Median survival (95% CI)
Age at diagnosis				
65-70	17/19	5.3 (2.6-7.9)	22/24	7.7 (6.1-9.3)
70-74	42/45	4.2 (2.2-6.2)	64/78	5.4 (3.3-7.5)
75-79	115/126	3.5 (2.9-4.0)	158/182	5.5 (4.5-6.4)
80-84	129/136	3.3 (2.6-3.9)	244/277	4.1 (3.5-4.7)
85-89	100/103	2.8 (2.4-3.3)	291/309	3.4 (3.1-3.7)
≥90	34/38	2.4 (1.5-3.3)	181/189	2.6 (2.3-3.0)
Overall	437/467	3.3 (3.0-3.5)	960/1059	3.9 (3.7-4.2)

Table 3. **Prognosis after diagnosis of dementia**, stratified by age and sex. Numbers reflect median life expectancy in years. N_t=number of deceased; N_{dem}=number of individuals with dementia; CI=confidence interval.

DISCUSSION

In this large population-based study we found that, of the overall life expectancy at age 65, on average 5% is spent with dementia, increasing to about 40% of remaining life expectancy at age 95, which varied by sex, educational attainment, and *APOE* genotype. The burden of disease in the population is highly amendable by preventive interventions that would postpone dementia onset by one to three years.

The added value of this study is the combination of the observed effects of sex, educational attainment, and *APOE* genotype on dementia incidence and mortality, evaluated in a well-defined population observed for a substantial period of time, and translated into population measures important to clinicians, patients, and policy makers in tackling the growing dementia epidemic. We found overall life expectancy and years lived with dementia similar to previous reports from France and Australia that were modelled using prevalence data.[13,14] Results from other studies have been more diverse. A US study of the Kaiser Permanente Medical Care Program in the 1970s and '80s found life expectancies with dementia at age 65 similar to ours, but at higher ages overall life expectancy was longer and less time lived with dementia,[11] possibly owing to a healthier population or the lower sensitivity of registry-based dementia diagnoses.[21] Another US study among community-dwelling individuals with in-person screening for dementia, reported higher, partly modelled, estimated years lived with dementia, along with longer overall life expectancy.[15] Both have been reported lower in Japan.[12] Differences in genetic make-up between populations and for example access to education may explain some of the discrepancies. Calendar time at which studies were performed also differed, along with methodological variation in use of prevalent versus incident data, simulations rather than direct observation, and a generally limited incorporation of comorbidity into the life year estimates.[11-15] What emerges from these studies jointly is an, on average, relatively low absolute number of remaining years lived with dementia, which nevertheless accounts for large shares of healthy life years lost for individual elderly patients, accompanied by an overwhelming burden on caregivers,[22] and health care expenditures that are substantially larger than those for other diseases.[23]

Importantly, a large share of the years lived with dementia in the population is amendable by preventive interventions at the population level. We show that interventions that succeed in postponing dementia onset by one to three years could result in 24-60% reductions in life years spent with dementia, which is in line with previously modelled reductions in the prevalence of Alzheimer's disease in the United States.[24] These percentages may somewhat attenuate when interventions simultaneously benefit overall life expectancy, but nevertheless illustrate that, at a population level, relatively minor gains in

dementia prevention may yield large benefits in public health, supporting the view that primary prevention has the largest effect on reduction of dementia occurrence and disability.[6] Moreover, given the estimated yearly care costs for dementia of nearly one trillion US dollars,[25] equating to around $50,000 per patient per year in the US,[26] such interventions would likely turn out cost-effective.[27] Given recently reported increases in life years spent with disability, much of which is likely attributable to cognitive impairment,[28] such preventive interventions promoting healthy ageing are paramount.

Higher educational attainment in our study related to lower shares of total life expectancy lived with dementia, in line with findings for individuals with at least college education in the U.S.[15] Although this may in part be attributable to for instance lifestyle differences, it fits well with the concept of cognitive reserve, reflecting the idea that higher educational attainment is able to mitigate the impact of brain pathology on clinical symptoms.[29] However, this would also imply increased mortality after diagnosis of dementia in highly educated individuals, for which we, similar to a systematic review in 2009,[30] found no evidence in the present study. In any case, universal access to education and resolving associated lifestyle differences may warrant consideration in public health debate about dementia prevention. We additionally determined the effect of the *APOE* genotype on life expectancy with and without disease, which had not yet been studied before. Our findings indicate an especially large burden of disease in ε4 carriers, both in terms of the absolute number of years and the share of total life years lived with dementia. Whilst this may endorse the focused search for Alzheimer therapies in this group of individuals,[31] it should not delay preventive or curative efforts that are generalizable and therewith beneficial to the larger community.

Extensive data are available on prognosis after diagnosis of dementia, mostly from unselected populations.[32] Results, however, are rather heterogeneous,[7,10,32] with reported survival times ranging considerably from 1.8 to 7.2 years. This heterogeneity is largely attributable to age and sex make-up of the study population, and further caused by (vascular) comorbidity at time of diagnosis,[33] as well as methodological differences in dementia ascertainment, inclusion of prevalent versus incident cases, severity at time of diagnosis, follow-up time, and general life expectancy in the source population. Following postulated recommendations to overcome several methodological limitations,[32] we affirm that prognosis needs to be seen in particular in light of age at diagnosis, and likely also sex as a reflection of underlying survival differences in the population.

Strengths of the current study including its large, structured population-based design with long-term follow-up and meticulous case finding strategies for dementia, which limited

attrition to 2.2%. Certain potential limitations should also be considered. First, the embeddedness of the Rotterdam Study in the Dutch healthcare system with uniform access to care, and the predominantly Caucasian ethnicity of the study population may limit generalizability. Standards of care and overall life-expectancy in the Netherlands rank average when compared to other industrialized countries,[34] making our findings applicable to most of present day dementia related public health policy, notwithstanding the need for additional data from ethnically diverse populations, and low- to middle-income countries. Second, the study population consisted of individuals recruited during two consecutive recruitment waves of the Rotterdam Study, 10 years apart, and although we adjusted for year of birth in the analyses, certain cohort effects may persist. In particular, several studies have suggested a decline in the age-specific incidence of dementia over the past decades, potentially leading to an overestimation of the remaining lifetime spent with dementia. Third, the effects of education and *APOE* genotype might vary with age, which due to sample size restrictions was not accounted for in our analysis. Fourth, we did not have information about institutionalization of participants in our study, which could provide additional perspective for public health policy, as well as informal care provision and informing physicians and their patients.

In conclusion, the estimates presented here provide reliable and current information to facilitate public health policies on dementia care in face of the growing burden of disease. Moreover, they highlight the potential of preventive strategies at the population level to limit this burden in years to come.

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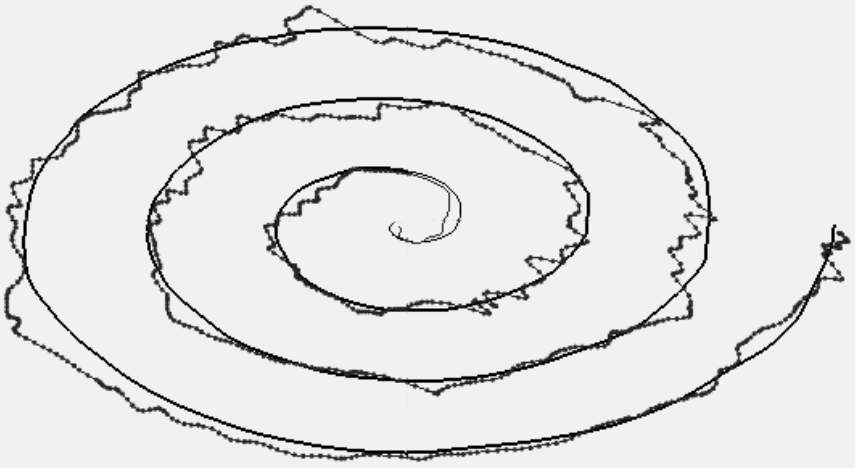
Chapter 2.2

Lifetime risk



ABSTRACT

Dementia, stroke, and parkinsonism are among the most common causes of neurological disability. Reliable estimates of lifetime risks of these common neurodegenerative and cerebrovascular conditions, including their co-occurrence, are important for projections of the societal burden of disease and healthcare resource allocation, but prospective life-course data of risk in the community is scarce. Within the prospective population-based Rotterdam Study, we studied lifetime risk of dementia, stroke, and parkinsonism between 1990 and 2016 in a competing risk framework. Among 12,102 individuals (58% women) aged ≥45 years who were free of these conditions at baseline, we studied co-occurrence, and quantified the combined, and outcome-specific lifetime risk of these conditions at various ages for men and women separately. In addition, we projected effects on lifetime risk of hypothetical preventive strategies that delay onset of these conditions by one, two and, three years, respectively. During 156,088 person-years of follow-up, 1,489 individuals were diagnosed with dementia, 1,243 with stroke, and 263 with parkinsonism. Of these individuals, 438 (14.6%) were diagnosed with multiple of these conditions. In total, 5,291 individuals died, of whom 3,260 remained free of dementia, stroke, and parkinsonism. The combined remaining lifetime risk at age 45 was 48.2% (95% confidence interval: 47.1-51.5) in women, and 36.2% (35.1-39.3) in men. This difference was attributable to a higher risk of dementia as the first manifesting condition in women than in men (25.9% versus 13.7%; *P*<0.001). Risk of stroke (19.0%) and parkinsonism (3.5%) were similar between men and women, although stroke occurred at younger ages in men. Women were almost twice as likely as men to be diagnosed with both stroke and dementia during their lifetime. Preventive strategies that delay disease onset with one to three years have the potential to reduce lifetime risk for developing any of these conditions by 20% to 50%. In conclusion, one in two women and one in three men will develop dementia, stroke, or parkinsonism during their lifespan. These findings strengthen the call for focus on preventive interventions at population level to reduce the burden of common neurodegenerative and cerebrovascular conditions in the ageing population.

INTRODUCTION

Dementia, stroke, and parkinsonism are among the leading causes of mortality and disability in older individuals.[1] Apart from their major impact on individual wellbeing, these common neurodegenerative and cerebrovascular conditions pose a significant social and economic burden on societies globally. Recent estimates indicate that the global costs-of-illness for these conditions sum up to more than 2% of the annual world gross domestic product.[2-4] The socio-economic burden is expected to grow steeply with the ageing of populations and continuing increases in life-expectancy worldwide.[1] This has led to widespread calls for prioritizing in particular stroke and dementia on the health agenda, with focus on prevention as the key to curbing this epidemic.[5-10]

However, despite the overwhelming concern for global health, dementia, stroke and parkinsonism remain understudied in terms of prevention at the population level,[11,12] and underfunded compared to other common high-burden conditions such as cancer and heart disease.[13,14] The difference in funding is most profound for charity, which likely reflects skewed perceptions of lifetime risk. Reliable and precise estimates of the burden of these common neurodegenerative and cerebrovascular conditions are needed as these may create awareness to the scope of the burden in the population. These estimates need to take into account the substantial co-occurrence of these conditions at old age,[15-18] a high competing risk of mortality, as well as potential differences in life-expectancy between men and women. Moreover, a comprehensible reflection of the high preventive potential,[5-10,19] in terms of lifetime risks, could further benefit public awareness and inform healthcare planning and resource allocation by policymakers alike.[1]

In this study, we therefore used long term follow-up data from the prospective population-based Rotterdam Study to calculate the combined, and outcome-specific lifetime risk of common neurodegenerative and cerebrovascular conditions within a competing risk framework in middle-aged and elderly men and women. We additionally studied their co-occurrence and preventive potential.

METHODS

Study population

This study was performed within the Rotterdam Study, a prospective population-based cohort designed to study the occurrence and determinants of age-related diseases in the general population. Details regarding the objectives and design have been reported previously.[15] Briefly, in 1990 all inhabitants aged 55 and older from a well-defined suburb in the city of Rotterdam, the Netherlands. I, received an invitation to participate. This initial cohort compromised 7,983 individuals. In 2000, 3,011 individuals who had become 55 years of age or moved into the study district since the start of the study were added to the cohort. In 2006, a further extension of the cohort was initiated in which 3,932 individuals were included, aged 45 years and older. In total, the Rotterdam Study comprises 14,926 individuals aged 45 years or over. The overall response rate for all three recruitment waves was 72%.

To estimate lifetime risk of these conditions, we excluded individuals with a history of these conditions at baseline (N=1,019: dementia [N=420], stroke [N=378], parkinsonism [N=106], or a combination hereof [N=115]), and we excluded individuals who were insufficiently screened at baseline for at least one of these conditions (N=1,780). We further excluded individuals who did not provide informed consent to access medical records (N=25), leaving 12,102 individuals for analyses. To study lifetime risk of each condition separately, we included all individuals who were free of that specific condition at baseline, underwent sufficient baseline screening and provided informed consent for follow-up (dementia: N=13,599, stroke: N=14,172, and parkinsonism: N=12,411).

Screening and surveillance for dementia, stroke, and parkinsonism

Baseline and follow-up ascertainment methods for dementia, stroke and parkinsonism have previously been described in detail.[7,16,17] At baseline, disease ascertainment comprised extensive structured interviews, examinations, and information from medical records, hospital discharge letters, and pharmacy data to ensure that participants were free of any of these conditions. During follow-up, we screened for all of these conditions during repeating examinations and interviews every four years. We additionally ensured continuous monitoring for disease through computerised linkage of medical records from general practitioners and the regional institute for outpatient mental healthcare with the study database. In the Dutch healthcare system, the entire population is entitled to primary care that is covered by their obligatory health insurance. The general practitioner functions as a gatekeeper for referral to secondary and tertiary care providers, who report back to the referring general practitioner about test results and clinical diagnoses. With this linkage, the

entire cohort is thus continuously monitored for detection of interval cases or possible clinical signs of these conditions between centre visits. Of all individuals who were screened and suspected of having any of these conditions, case reports were drawn up covering all potentially relevant information to establish the presence of disease. These case reports were evaluated by a consensus panel led by a consultant neurologist to adjudicate the final diagnosis in accordance with standardized diagnostic criteria, notably the DSM-III-R for all-cause dementia and NINCDS–ADRDA for Alzheimer's disease, WHO criteria for stroke, and strict study criteria for parkinsonism and Parkinson's disease.[17] In addition, available clinical neuroimaging data were used if required to determine diagnosis subtype. Study follow-up ended at date of incident outcome diagnosis, death, or 1st January 2016, whichever came first, and was completed for 96.2% of potential person-years.

Other measurements

Standardized assessment of anthropometrics, risk factors, and use of medication at baseline through in-person examinations at the research centre, home interviews, and laboratory assessments has previously been described in detail.[15] During home interviews, educational attainment were assessed and categorized as primary education, lower/intermediate general education or lower vocational education, intermediate vocational education or higher general education, and higher vocational education or university. Smoking habits were assessed during the same interviews, and subsequently classified into current, former and never smokers. Blood pressure was measured twice in sitting position on the right arm using a random-zero sphygmomanometer, and the average of two measurements was used for analysis. Depressive symptoms were assessed by using the Center for Epidemiology Depression Scale (CES-D), with a score ≥16 considered suggestive of depressive symptoms. Atrial fibrillation was diagnosed on ECG, and participants were continuously monitored with linkage of medical records. Diabetes was defined as a random serum glucose concentration ≥11.1 mmol/L, a fasting serum glucoseconcentration ≥7.0 mmol/L, or the use of blood glucose lowering medication. *APOE* genotype was obtained using polymerase chain reaction of coded DNA samples in the original cohort, and with a bi-allelic TaqMan assay in the extended cohort.

Analysis

Preclusion of disease-specific outcomes of interest by death or other outcomes are referred to as competing risks.[20-22] Due to these competing events, absolute risks are overestimated in standard Kaplan-Meier analyses. Since women on average live longer than men, this overestimation will be differential. To overcome these issues, we analyzed the data taking into account the occurrence of competing events to compute lifetime risk in left truncated data with age as time scale. Lifetime risk estimates reflect the competing risk-adjusted

cumulative incidences from that particular age to the age of last observation. In this study, the highest age was 106 years for men, and 107 for women.

We stratified by sex in all analyses given a substantial difference in life-expectancy between men and women. First, we assessed the combined and condition-specific cumulative incidence of these conditions from 45 years old up until a certain age. In condition-specific analyses, individuals were at risk of all conditions irrespective of the occurrence of precluding events, e.g. individuals with a stroke during follow-up remained at risk for dementia. We studied their co-occurrence in more detail by quantifying the number of individuals who developed more than one of these conditions during follow-up among individuals who were free of each of these conditions at baseline. We subsequently computed the combined remaining lifetime risk of developing any of these conditions at index ages 45, 55, 65, 75, and 85 while taking into account their co-occurrence.

Next, we quantified the remaining lifetime risk of first manifestation of each condition separately (all-cause dementia, all-cause stroke (ischemic and hemorrhagic), and all-cause parkinsonism) in individuals who were free of each of these conditions at index ages 45, 55, 65, 75, and 85. We additionally quantified to what extent each condition contributed to the combined risk of developing any of these conditions. Additionally, we calculated the corresponding lifetime risk of multiple common neurologic conditions, doing so for each possible combination separately. In stratified analyses, we also computed lifetime risks for each condition separately and we compared sex-differences in cumulative incidence and remaining lifetime risk of the most common subtypes for each condition separately (Alzheimer's disease, ischemic, hemorrhagic and unspecified stroke and Parkinson's disease). For each of these analyses, we selected all individuals free of each condition or subtype of interest at baseline.

Finally, we modelled the effects of a delay in disease onset on the combined lifetime risk of any condition and for each condition separately at index ages 45, 55 ,65 ,75, and 85 by postponing the date of diagnosis of all three conditions with 1, 2, and 3 years. In these analyses, we assumed a constant life-expectancy.

We used nominal significance levels to compare age and sex differences (α=0.05). Data were handled and analysed with SPSS Statistics version 24.0 (IBM Corp., Armonk, NY) and R version 3.4.3 (rms, etm, and cmprsk packages).

RESULTS

Population characteristics are presented in Table 1. Median age at baseline was 62.2 years (range 45-107 years), and women represented 57.7% of the population. During 156,088 person-years of follow-up, 3,076 individuals developed one or more of these conditions: 1,489 individuals were diagnosed with dementia (79.7% Alzheimer's disease), 1,285 with stroke (64.7% ischaemic, 9.8% haemorrhagic stroke, 25.4% unspecified) and 263 with parkinsonism (50.6% Parkinson's disease). Among those individuals diagnosed with one of these neurological conditions, 438 (14.6%) individuals were diagnosed with more than one condition. In total, 5,291 individuals died during follow-up, of whom 3,260 died free of these conditions.

Characteristics	**All individuals** (*N*=12,102)	**Men** (*N*=5,120)	**Women** (*N*=6,982)
Age, years	64.4 (±9.4)	63.8 (±8.8)	64.9 (±9.8)
Educational attainment			
Primary	1943 (16.1%)	577 (11.3%)	1366 (19.6%)
Lower	4820 (39.8%)	1494 (29.2%)	3326 (47.6%)
Further	3306 (27.3%)	1844 (36.0%)	1462 (20.9%)
Higher	1848 (15.3%)	1144 (22.3%)	704 (10.1%)
Smoking			
Never	3164 (26.1%)	656 (11.8%)	2508 (35.9%)
Past	4689 (38.7%)	2513 (49.1%)	2176 (31.2%)
Current	2217 (18.3%)	1120 (21.9%)	1097 (15.7%)
Systolic blood pressure, mmHg	139 (±21)	141 (±20)	138 (±22)
Depression	801 (6.6%)	195 (3.8%)	606 (8.7%)
Atrial fibrillation	599 (4.9%)	309 (6.0%)	290 (4.2%)
Diabetes	1124 (9.3%)	574 (11.2%)	550 (7.9%)
APOE genotype			
ε2/ε2 or ε2/ε3	1510 (12.5%)	605 (11.8%)	905 (13.0%)
ε3/ε3	6675 (55.2%)	2874 (56.1%)	3801 (54.4%)
ε2/ε4, ε3/ε4, or ε4/ε4	3239 (26.8%)	1400 (27.3%)	1839 (26.4%)

Table 1. Baseline characteristics of individuals free of stroke, dementia, and parkinsonism**.** Data are presented as frequency (%) for categorical, and mean±standard deviation for continuous variables.

Risk of specific conditions across the age span

In Figure 1, the combined and condition-specific risk from the age of 45 until various ages is presented for women and men separately. These risks increased steeply with age, for both the combined and outcome-specific incidence. For dementia for instance, this ranged from 0.4% for women and 0.2% for men aged 45 years until age 65, to up to 29.2% and 18.4% until the age 95.

Lifetime risk of any condition

Among all individuals free of dementia, stroke and parkinsonism at baseline (N=12,102), there were 2,571 primary first diagnoses: dementia was the first manifestation in 1,245 individuals, in 1,118 individuals this was a stroke, and 208 individuals developed parkinsonism as first manifestation. In Figure 2, lifetime risks of developing dementia, stroke, and parkinsonism are shown at different ages for men and women separately. For instance, a 45-year old woman had a 48.2% risk of developing any of these conditions during her remaining lifetime, while for a 45-year-old man this risk was 36.3% (*P*-value for sex difference <0.001).

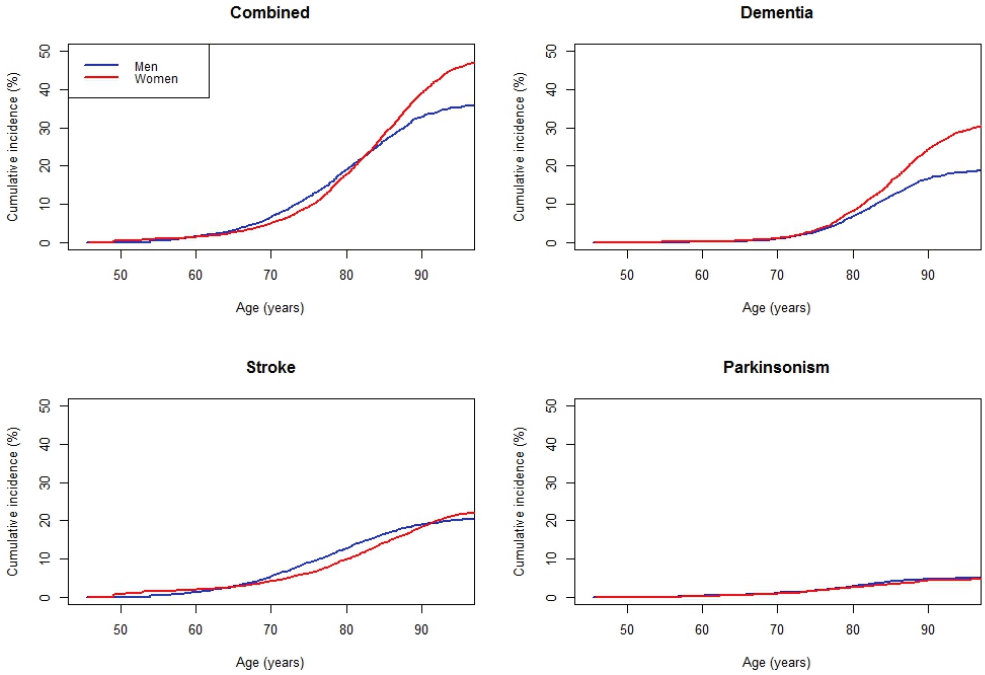


Figure 1. Risk of common neurodegenerative and cerebrovascular conditions for men and women.

Among those who develop dementia, stroke, or parkinsonism during their lifetime, most individuals will be diagnosed with only one of these conditions (Figure 3, Panel A). Yet, there is a substantial risk at the age of 45 to face multiple conditions during the remaining lifespan. Women at the age of 45 are more likely to be diagnosed with more than one of these conditions during their remaining lifetime compared to men (Figure 3, Panel A: 10.1% compared to 8.2% respectively, *P*<0.001). This difference was predominantly driven by a greater probability of overlap between dementia and stroke (5.9% in women compared to 3.6% in men, *P*<0.001). Similar to lifetime risks of any of these conditions, lifetime risk of developing multiple conditions remained stable until the age of 85 (Figure 3, Panel B).

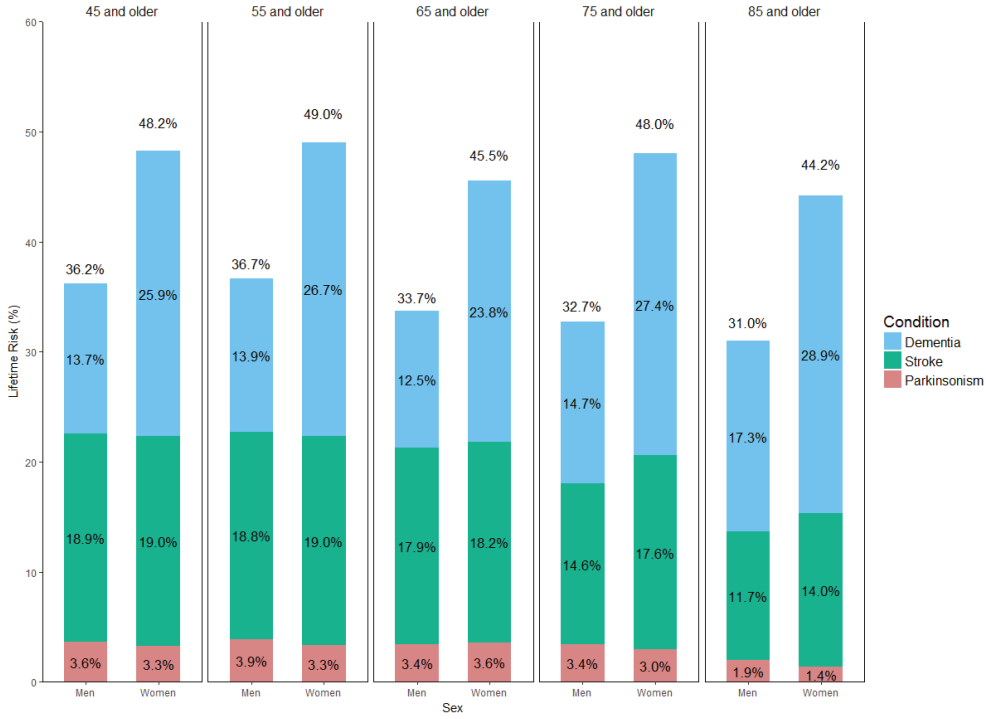


Figure 2. Remaining lifetime risk of first manifestation of common neurodegenerative and cerebrovascular conditions at different ages, stratified by sex.

Lifetime risk across the age span: first manifestation only

Lifetime risk estimates of first manifestation of common neurologic conditions were stable for both men and women between ages 45 and 85 (Figure 3). At age 45, first manifestation of stroke posed the highest lifetime risk for men (18.9%). Dementia posed the largest risk for women (25.9%), which was significantly higher than in men (13.7%; $P<0.001$). This sex difference remained largely stable across all index ages ($P<0.001$ for all ages). Figure 3 also shows that with advancing age, the relative contribution of dementia to remaining lifetime risk of any condition increased in both men and women, representing 66.6% of all first diagnoses in elderly women (i.e. >85 years) and 55.6% in elderly men. For stroke, men and women had similar lifetime risk at the age of 45 (18.9% in men compared to 19.0% in women, $P=0.46$). However, men were at substantially higher risk of developing stroke at younger ages, such that they have a 8.4% risk of developing stroke before the age of 75 years compared to 5.8% for women ($P=0.005$). Lifetime risk of parkinsonism peaked earlier compared to dementia and stroke, was low after 85 years, and not significantly different between men and women at any age ($P>0.16$).

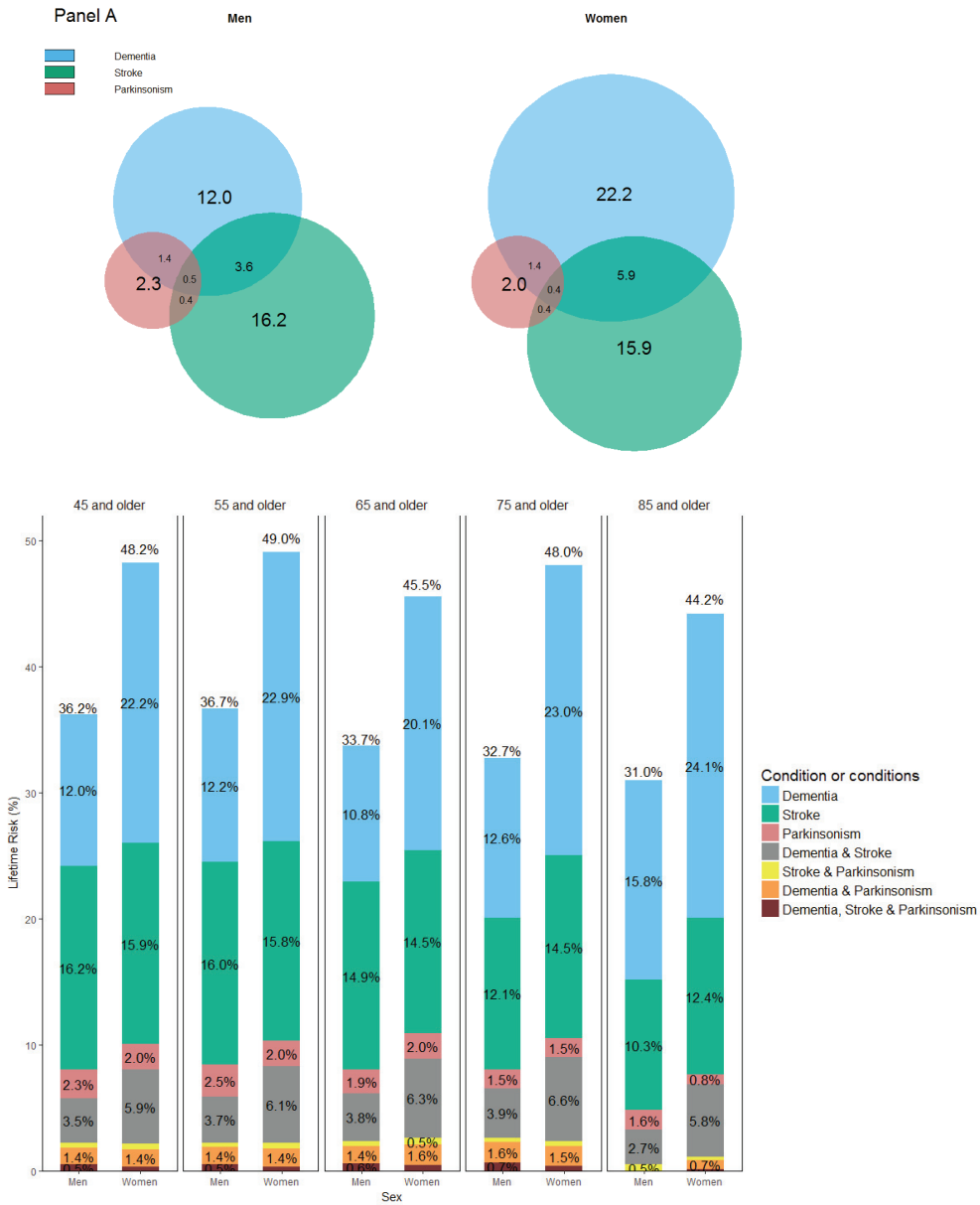


Figure 3. Lifetime risk of multiple conditions from the age of 45 onwards in men and women (Panel A, top), and remaining sex-specific lifetime risk of common neurodegenerative and cerebrovascular diseases and its possible combinations by age (Panel B, bottom). For instance, a woman aged 45 years has a 5.9% risk to develop both dementia and a stroke, and 0.4% risk of all three conditions during her remaining lifetime.

Lifetime risk of each condition separately

Among individuals free of each condition of interest at baseline, women aged 45 years had a significantly higher lifetime risk of developing dementia than men (31.6% compared to 19.1% in men; *P*<0.001), whereas lifetime risk of stroke (22.4% in women and 20.7% in men, *P*=0.12) and parkinsonism (4.7% in women and 4.9% in men, *P*=0.27) were not significantly different. In sex-stratified analyses, we observed similar patterns for Alzheimer's disease, ischemic and hemorrhagic stroke and Parkinson's disease (data not shown).

Projecting a delay in disease onset and occurrence

When projecting a delay in disease onset of one, two or three years for all three conditions, the remaining lifetime risk common neurodegenerative and cerebrovascular conditions could be reduced by 20% in individuals aged 45 years and older, and by more than 50% in the oldest of old (Figure 4). Even a delay in onset for a few years of only one condition, could already result in substantial reductions for the combined lifetime risk of developing any of these conditions. For instance, delaying dementia onset with 3 years, has the potential to relatively reduce lifetime risk of any common neurodegenerative and cerebrovascular condition with 15% for men and women aged 45, up to 30% for those aged 85 years and older. For a 85-year old woman, this lowers the risk of developing dementia during her remaining lifetime from 30.4% to 21.3%.

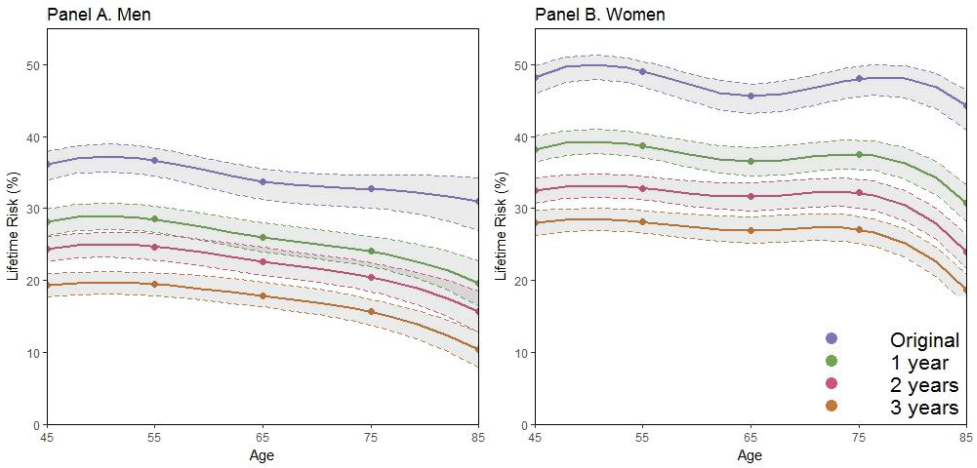


Figure 4. The effect of preventive interventions that delay the onset or occurrence of disease. Modelling the effect of a 1, 2, and 3-year delayed onset of disease on the combined remaining lifetime risk of developing dementia, stroke or parkinsonism in men (Panel A) and women (Panel B) at ages 45, 55 ,65 ,75, and 85 and older. In these analyses, date of diagnosis of all three conditions was delayed with 1, 2, or 3 years while assuming a constant life-expectancy. Areas in shaded grey represent 95% confidence intervals.

DISCUSSION

In this long-term population-based study, we found that one in two women develop dementia, stroke, or parkinsonism during their lifetime. For men, this risk approximates one in three. There is substantial overlap between these diseases, in particular in women who are almost twice as likely as men to be diagnosed with both stroke and dementia during their lifetime. Preventive strategies that delay disease onset of all three conditions by one to three years have the potential to reduce risks by 20 to 50%. These findings strengthen the call for focus on prevention to reduce the current and projected global burden of common neurological conditions in the ageing population.

Prior studies have generally quantified the burden of stroke, dementia, and parkinsonism separately,[23-28] or based on prevalence or incidence rates without accounting for their potential co-occurrence and competing mortality due to other causes.[1] This pattern of co-occurrence and competing mortality hampers reliable calculation of the risk of developing any of these conditions when applying a lifetime perspective, such that the occurrence of one condition (e.g. stroke) precludes consideration of any subsequent event (e.g., post-stroke dementia). Similarly, several risk factors (e.g. hypertension) not only increase the susceptibility for these common neurodegenerative and cerebrovascular conditions, but are also associated with an increased risk of dying from other causes (e.g. heart disease).[29] Previously, the Framingham Heart Study reported lifetime risks of both dementia (1 in 5 women, 1 in 10 men),[25,28] and stroke (1 in 5 women, 1 in 6 men),[23,30] as well as a combined estimate of those two assessed in a single study (1 in 3).[31] Compared to findings from that study, we observed higher a lifetime risk of dementia (1 in 3 women, 1 in 5 men). This discrepancy may be due to the fact that individuals in the Rotterdam Study, have a slightly longer life-expectancy (the Netherlands, women 83.5 and men 81.7 years) compared to individuals in the Framingham Heart Study (US, women 81.2 and men 76.4 years). Apart from a longer life-expectancy in general, these findings may be explained by smaller differences in life-expectancy between men and women in the Netherlands (1.8 years), compared to the US (4.8 years). With longer life-expectancy, individuals in this study simply had more time to develop these conditions in a timeframe with high age-specific incidence rates.[32] In line with estimates from the Framingham Heart Study, we found similar lifetime risks of stroke for both men and women. Due to differences in risk factor distributions, men have nevertheless a greater propensity of developing stroke at younger ages.[33] For parkinsonism and Parkinson's disease, we found similar lifetime risks for men and women, corroborating evidence from a previous study.[26] In contrast, we did find lower risks of Parkinson's disease for men (2.9%), compared to the lifetime risk of 6.7% reported in another study solely conducted in male physicians. Physicians may be more aware of their

changing health (e.g., symptom recognition), which may help explain this higher risk. In addition, this discrepancy may be due to distinct differences in baseline characteristics, such as a lower history of ever-smoking (50% compared to 88% in this study), a factor that is strongly, yet inversely, associated with Parkinson's disease.[27]

Lifetime risk estimates can be used to effectively inform policy makers and communicate risks to the general population, given their easier interpretation compared with measures such as incidence, prevalence, or relative risk.[34,35] These approaches to raise awareness and inform the public through lifetime risk estimates have been successfully implemented for other diseases, such as breast cancer or heart disease.[36,37] Nowadays, preventive measures for primary prevention of cardiovascular disease are tailored to individual lifetime risk estimates.[38,39] This could inform future prevention programs for common neurologic conditions.

There are currently no disease modifying drugs available for dementia and most causes of parkinsonism, and prevention of stroke is hampered by suboptimal adherence to effective preventive strategies or unmet guideline thresholds.[40,41] Delay in onset of these common neurodegenerative and cerebrovascular conditions by merely a few years could reduce the population burden of these diseases substantially. However, preventive interventions may also contribute to a drop in competing mortality by affecting lifetime risk of diseases with shared risk factors, such as coronary heart disease and peripheral artery disease. Our projections of the preventive potential, which assumed constant life expectancy, may therefore be overestimating the compression of morbidity. Nevertheless, they illustrate that risks could drop strikingly with relatively minor delays in the occurrence and onset of disease, underlining the importance of preventive strategies as the way forward to combat these diseases on a global scale.[3,42-45] These results also have implications for the type of prevention strategies to develop, including population-wide interventions targeted at risk factors with considerable sex-specific differences in attributable risk, such as loneliness and depression in women or diet in men.[10,43]

Several methodological considerations should be taken into account when interpreting these lifetime risks. First, this population-based study predominantly included individuals of European ancestry (97%). Generalizing these lifetime risk estimates to other ethnicities should be done with caution. Second, although the overall response rate in the Rotterdam Study was high (72%), non-responders and individuals with insufficient screening at study entry may have had higher than average risk factor burden and associated risk of these diseases, which may have led to some underestimation of lifetime risks.[46] Finally, we were unable to take into account severity of clinical disease, and in particular for interpretation of

stroke estimates, it should be noted that about half of ischemic strokes at the population level classify as minor according to the NIH Stroke Scale (NIHSS) and may have a limited impact on daily life.[47]

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

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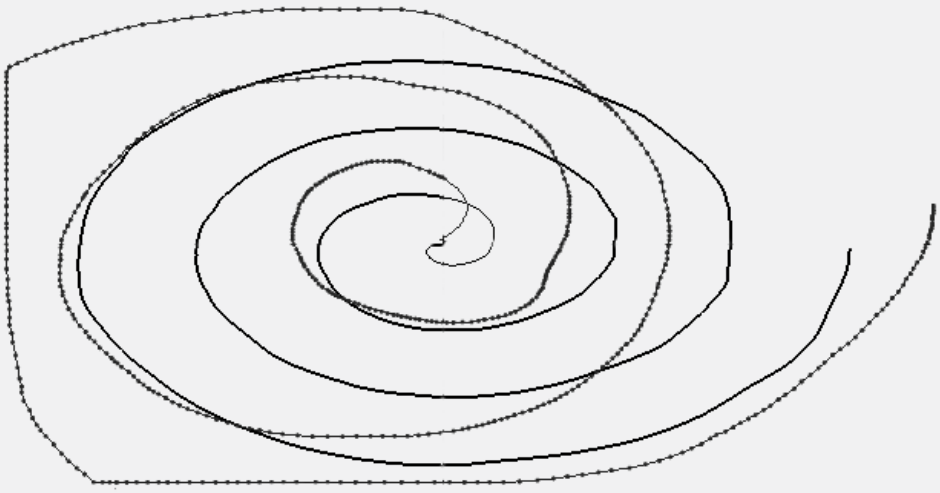
Chapter 2.3

Time trends in the incidence



ABSTRACT

Several studies have recently reported a decline in the incidence of dementia by up to 20% per decade, which may have large implications for the projected burden of disease, and provide important guidance to preventive efforts against dementia. However, individual studies are often hampered by limited sample size, and have therefore not been able to provide conclusive results regarding sex differences, and underlying causes. We aggregated data from 7 population-based cohorts from the United States and Europe to determine changes in the incidence of dementia since 1990. Included cohorts are the Framingham Heart Study, the AGES-Reykjavik Study, the Rotterdam Study, the Gothenburg studies, the Three-City Study, the Personnes Agées Quid study, and the Cognitive Function and Ageing Studies. We first calculated age- and sex-specific incidence rates per study, and then defined non-overlapping 5-year epochs within each study to determine within study trends in incidence. Estimates of change per 10-year interval were pooled using fixed effects meta-analysis. Of 46,976 individuals (60% women), 4,719 developed dementia in the 5-year epochs. The incidence of dementia increased steeply with age, similar across studies, from about 5 per 1000 person years in individuals aged 65-69, to roughly 60 per 1,000 person years for those aged 85-89. The hazard of dementia consistently declined with on average 17% per decade (95% confidence interval: 9-24%; I^2=21% [0-66%]). Estimates were similar for Alzheimer's Disease only (17% [8-24%]), and did not materially differ between men and women. In conclusion, the incidence of dementia in Europe and North America has declined by 17% per decade over the past 25 years, consistent across available studies and similar for men and women. Within this collaboration we further seek potential causes of these trends, including educational attainment, and cardiovascular risk profile.

INTRODUCTION

At present, an estimated 48 million people worldwide are living with dementia, making it a leading cause of dependence and disability. Because of a rapidly aging population, this number will nearly double in the next 20 years, and the socioeconomic burden of dementia will increase accordingly. It has led to widespread calls for global action against dementia, with the aim of finding a disease-modifying therapy by 2025.[1,2] While the majority of research efforts are targeted at identifying pathophysiological mechanisms and drug discovery at a biological level, the full spectrum of dementia is arguably better captured in a life-course perspective across populations.[3] Projections of the burden of dementia could be alleviated if improvements in life conditions and health care over the last decades have had beneficial effects on dementia risk in the population. Indeed, recent studies in North America and Europe have reported a decline in the incidence of dementia over the last fourty years, with reductions of 10% to 38% per decade,[4-7] potentially able to offset effects of the ageing population.[6-8] However, reliable projections for the future burden of dementia depend on the precise magnitude of decline, and prolongation of trends into the coming decades, for which insight into sex differences, ethnic variation, and the underlying causes is essential.[8]

Reliable assessment of time trends in the incidence of dementia calls for careful monitoring in the general population, in a consistent manner over a prolonged period of time. Population-based cohort studies are generally designed with this aim in mind, but relatively few have collected data on dementia incidence spanning multiple decades. Moreover, these individual studies at times lack precision to address the various research questions outlined above. We therefore aggregated data from available long-term population-based studies in Europe and the United States of America (USA) to seek confirmation of a downward trend in dementia incidence, determine the magnitude of any trend, and establish whether changes were observed in men and women in a similar way.

METHODS

The Alzheimer Cohorts Consortium

The Alzheimer Cohorts Consortium is composed of nine population-based cohorts that have prospectively collected data on dementia in addition to genotyping and extensive phenotyping, particularly in terms of cardiovascular factors and brain magnetic resonance imaging (MRI). The participating cohorts are located in the USA, Europe, and Scandinavia, and include the Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study, the

Atherosclerosis Risk in Communities (ARIC) study, the Cardiovascular Health Study (CHS), the Cognitive Function and Ageing Studies (CFAS), the Framingham Heart Study (FHS), the Gothenburg population studies, the Personnes Agées QUID (PAQUID) study, the Rotterdam Study, and the Three-City Study (3C). More detailed information on the Alzheimer Cohorts Consortium has been published previously.[9]

Cohorts

The present study included the seven of nine participating cohorts that had sufficient follow-up information available to consistently assess incidence rates, and determine prolonged time-trends in dementia incidence. Participating cohorts and data collection summaries are presented in Table 1, and include a total of more than 70,000 individuals of whom around 6,300 have developed dementia to date. Briefly, the **Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study** represents a sample drawn from the population-based Reykjavik Study cohort.[10] The original Reykjavik Study comprised a random sample of 30,795 men and women born between 1907 and 1935 and living in Reykjavik in 1967. Between 1967 and 1996, six examinations were conducted in six sub-cohorts, and 5764 survivors of the original cohort were re-examined for the AGES-Reykjavik study between 2002 and 2006. The **Cognitive Function and Ageing Studies (CFAS)** comprise two three-site population-based studies among individuals aged 65 years and over living in the community, including those in institutions.[6] The original study began in 1989 (CFAS I), and a comparison study with independen sampling was initiated two decades later, with baseline interviewing undertaken from 2008-2010 (CFAS II). In 2011 the CFAS Wales study began. The **Framingham Heart Study (FHS)** began in 1948 with the recruitment of an original cohort of 5,209 men and women who were 28 to 62 years of age at entry.[11] In 1971, a second generation of study participants, including 5,124 children and spouses of children of the original cohort were enrolled.[12] Enrolment of the third generation cohort of 4,095 children of offspring cohort participants began in 2002.[13] The **Gothenburg population studies** consist of four studies among individuals representative of the Swedish population.[14,15] These include Kvinnoundersökningen (KVUS), a study of 1,462 women aged 38-60 who are followed since 1968; the H70 study, which studies several birth cohorts of 70-year olds recruited in 1971; the H85 study, which started in 1986 with enrolment of a birth cohort of 85-year olds; and the 95-plus study that started in 1996 and by 2012 had recruited a total of 950 individuals. The **Personnes Agées QUID (PAQUID)** cohort is a population-based study in the southwest of France among 3,777 individuals aged 65 years or older recruited in 1988.[16] There have been nine subsequent waves of data collection at 1, 3, 5, 8, 10, 13, 15, 17, and 20 years after the baseline assessment. The **Rotterdam Study (RS)** is a prospective population-based cohort study comprising 14,926 subjects aged 45 years or older.[17] Baseline data of 7,983 participants were collected between 1990–1993, with subsequent cohort expansions in 2000

(3,011 individuals) and 2006 (3,932 individuals). Participants are interviewed at home and re-examined at a dedicated research center once every four years. In addition, the entire cohort is continuously under surveillance for disease outcomes through linkage of electronic medical records with the study database. The **Three-City Study (3C)** is a longitudinal population-based study of the relation between vascular diseases and dementia in persons aged 65 years and older.[18] Between 1999 and 2001, a total of 9,294 non-institutionalized persons were recruited from the electoral rolls of three French cities: Bordeaux (South-West), Dijon (North-East) and Montpellier (South-East). Extensive follow-up examinations were performed every two years after the baseline assessment, comprising standardised questionnaires, clinical examinations, and detailed cognitive assessment. All of the participating studies were approved by their respective institutional review committee, and all subjects provided written informed consent.

Assessment of dementia and Alzheimer's disease

Our primary outcome of interest is all-cause dementia with a secondary outcome of diagnosis of Alzheimer's Disease, where available. Diagnosis criteria for all cohorts in the Alzheimer's Cohort Consortium are listed in Table 1. For those cohorts included in these analyses, diagnosis of dementia was based on either DSM-IIIR (CFAS, Gothenburg studies, PAQUID and the Rotterdam study) or DSM-IV (AGES, FHS and 3C). The NINCDS-ADRDA criteria for Alzheimer's diagnosis was used in all cohorts except CFAS which does not have data on Alzheimer's diagnoses.

Analysis

Means and SD are presented for continuous variables and frequencies and relative frequencies are presented for categorical variables. Poisson regression was used to calculate 5-year incidence rates (IRs) and 95% confidence intervals, adjusting for age and sex (for non sex specific models) and using the log of follow-up time as an offset variable and presented for the middle age of each age group. All cohorts have repeated measures for participants, so a single participant can contribute to multiple age groups if they are free from dementia at the start of the age-group category. A robust sandwich estimator was used to calculate the 95% confidence interval in order to control for the violation of the independence assumption.[19]

Cox proportional hazard regression models, were used to calculate the five-year cumulative hazards and hazard ratios (HR) for all-cause dementia and Alzheimer's disease. Additionally, models stratified by age groups and sex were performed for all-cause dementia. Cohort-specific, non-overlapping epochs were created in order to maximize the person-years available in each cohort. All models were adjusted for age at entry of the epoch and sex, if

applicable. Participants were included in an epoch if free of dementia at the beginning of the epoch, and followed for onset of dementia in the next five years. Participants were censored at time of dementia diagnosis, death, or at the end of the five-year follow-up, whichever came first. Similar to the incidence analyses, participants can contribute to multiple epochs if they were free of dementia at the beginning of the epoch with a robust sandwich estimator for the covariance structure to estimate the 95% confidence limits.[19] Five-year cumulative hazards are calculated for a person aged 75 and presented as rates per 100 persons. Hazard ratios are calculated for each epoch relative to the first epoch. Since epochs are specific to each cohort, to explore temporal trends we calculated a hazard ratio per ten years change, which was then meta-analysed across all cohorts using random effects in the "meta"-package (version 4.8-4) of the statistical software R, version 3.4.2. This is interpreted as a change in five-year hazard per decade advance in calendar time and was estimated using years from the median start date of each epoch, divided by ten and treated as a continuous variable in the model. The AGES cohort were not included in the trends analyses, because of insufficient examination cycles to define within study epochs.

Finally, we extrapolated the meta-analysed trend per decade to worldwide dementia incidence estimates until 2040. We took global estimates of 2010 and 2015 incidence from the WHO and World Alzheimer reports.[20,21] We assumed continuation of an overall 25% increase in the incidence per five years (due to population ageing), and calculated the change in the expected absolute number of new cases within each five-year time-window until 2040 if observed trends in the incidence would be extended globally over the next two decades. For region-specific assessment (i.e. Europe and North America), we assumed that the share of total cases originating in these regions would continue to fall by 3% per five years.[20,21]

All analyses were done separately by investigators at each cohort. In order to ensure harmony in analyses, each cohort received a detailed analysis plan, including statistical programs in both SPSS (IBM Corp., Armonk, NY) and SAS (SAS Institute, Cary, NC).

RESULTS

Cohort characteristics, and demographics of participants in the analyses per cohort are presented in Table 1. Data on over 55,000 participants with between 2 to 27 years of follow-up are included in this study. All the cohorts are made up of more females than males with the relative frequency ranging from 56.8% in FHS to 76.3 in the Gothenburg studies. Mean ages are also similar across cohorts, all between 72 and 77.

A total of 46,229 individuals, 41% male, were included in the incidence analyses followed for a total of almost 289,998 person-years. We observed a total of 4,473 incident cases of dementia with 1,410 (32%) males (Table 1). We saw consistent incidence rates by age group across all cohorts (Figure 1). Age- and sex-adjusted incidence of dementia increased with age, ranging from 2.5 to 8.6 per 1000 person-years in the youngest age group (65-69 years), and from 37.7 to 69.3 per 1,000 person-years in the oldest participants for whom ample data was available (85-89 years). In general, the CFAS II cohort had the lowest incidence rates and PAQUID had the highest. This pattern was similar for the sex-specific results (Table 2).

Cohorts with sufficient follow-up data to create at least two epochs were included in the analysis of trends over the last 25 years. Epochs were created specific to each cohorts design, with two epochs in the Three-Cities study, three epochs in PAQUID and the Rotterdam study, four epochs in FHS, and five epochs in the Gothenburg studies. We directly compared and meta-analysed the 5-years hazard ratios per 10-year increment in calendar time between cohorts. This showed a consistent decrease in the five-year cumulative hazard of all-cause dementia in all cohorts (Figure 2). When meta-analysing across all studies, we see an 17% (95% confidence interval: 9-24%) decrease in all-cause dementia per decade since 1990, similar for Alzheimer's disease (Figure 2). This decrease was seen for men as well as women (HR 0.78 [0.68-0.88] versus 0.89 [0.81-0.98], respectively), and broadly similar across age groups (data not shown).

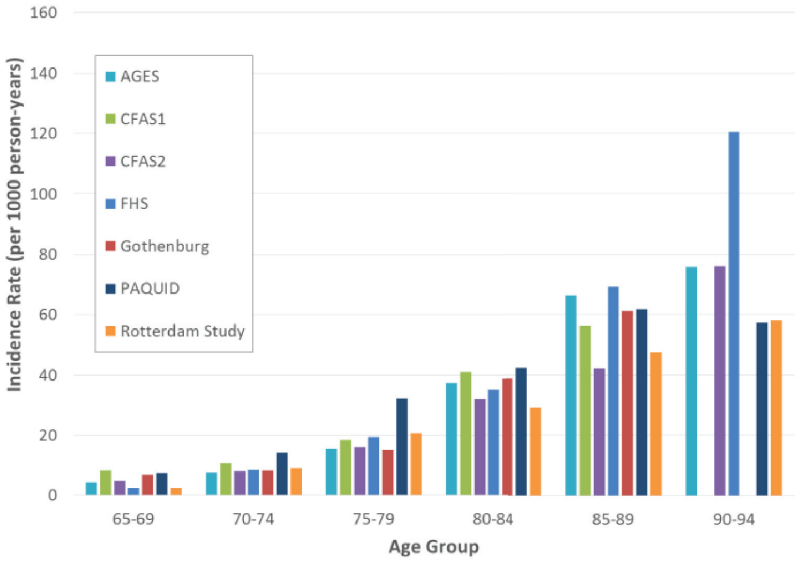


Figure 1. The age-specific incidence rate of dementia by study.

Study	AGES-Reykjavik	CFAS I/II	Framingham Heart Study	Gothenburg Studies	PAQUID	Rotterdam Study	Three-City Study
Country	Iceland	UK	USA	Sweden	France	Netherlands	France
Study baseline	2002	1991/2008	1990	1990	1988	1990	1999
Study sites	1	3 / 3	1	1	1	1	3
At risk of dementia	5,722	7,635 / 7,762	8,586	3,024	2,997	11,044	8,250
Dementia follow-up, y	14	4 / 2	25	25	27	25	16
Mean age, y	77	75.0 / 76.4	72.1	77.3	75.3	72	74
Women, %	57.7%	61.6% / 56.1%	56.8%	76.3%	58.0%	58.5%	61.3%
Caucasian ethnicity, %	100%	99.1% / 97.2%	100%	100%	N/A	98.0%	100%
Dementia diagnosis							
All-cause dementia	DSM-IV	DSM-IIIR	DSM-IV	DSM-IIIR	DSM-IIIR	DSM-IIIR	DSM-IV
Incident cases	250	250 / 250	800	700	940	1,400	950
Alzheimer's disease	NINCDS-ADRDA	N/A	NINCDS-ADRDA	NINCDS-ADRDA	NINCDS-ADRDA	NINCDS-ADRDA	NINCDS-ADRDA
Incident cases	150	N/A	510	300	730	1,100	650

Table 1. Demographics and characteristics of cohorts and participants. N/A=not available; y=years.

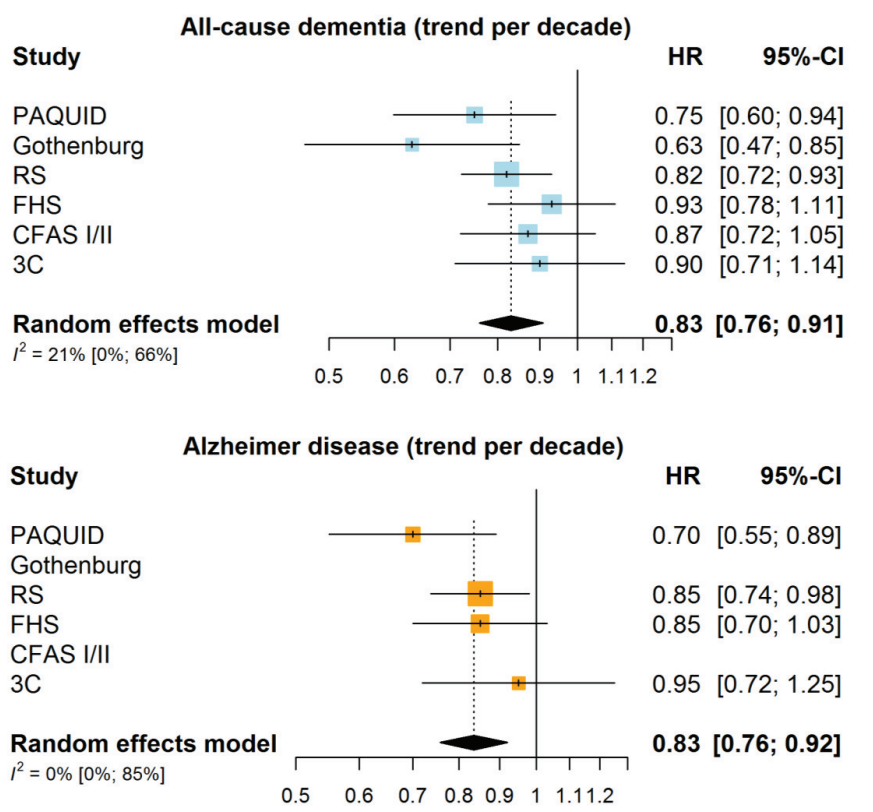


Figure 2. Change in the incidence of dementia. Trends are expressed as change per decade for each study separately, and combined in a meta-analysis.

DISCUSSION

The age-adjusted incidence of dementia in Europe and North America has declined by 17% per decade over the past 25 years, consistent across available studies, and similar for men and women. This trend extended to clinical Alzheimer's disease with a decrease of 17% per decade. Assuming continuation of this trend in Europe and North America into the coming decades, this could mean 20 million people less will develop dementia by 2040, compared to widely upheld projections of the global burden of disease.[21] If the same incidence reduction could be achieved worldwide, this could lead to a reduction of up to 150 million new cases of dementia by 2040.

	AGES Reykjavik	CFAS I	CFAS II	Framingham Heart Study	Gothenburg Studies	PAQUID	Rotterdam Study
Overall population							
Sample Size	5764	6441	11788	2596	3024	2960	13656
Person-Years	23970	12850	25319	29906	29364	19631	148958
Dementia	477	261	390	685	612	437	1611
Age Groups							
65-69	4.5 (2.7-6.5)	8.6 (5.2-14.2)	5.41 (2.6-11.3)	2.5 (1.4-4.6)	7.1 (4.1-12.3)	7.7 (5.3-11.3)	2.5 (1.6-3.8)
70-74	7.9 (6.4-9.5)	11.0 (6.8-17.7)	10.1 (5.8-17.6)	8.6 (6.6-11.2)	8.5 (5.3-13.9)	14.3 (9.7-21.1)	9.2 (7.2-11.7)
75-79	15.7 (13.4-18.1)	18.6 (11.7-29.4)	13.6 (7.0-26.6)	19.6 (16.6-23.2)	15.3 (7.7-30.2)	32.3 (25.0-41.7)	20.9 (17.3-25.2)
80-84	37.3 (33.3-41.5)	41.2 (29.3-57.9)	22.3 (12.7-38.9)	35.2 (30.5-40.6)	38.9 (17.2-87.9)	42.4 (30.8-58.4)	29.2 (23.7-36)
85-89	66.3 (56.3-76.8)	56.3 (38.9-81.4)	37.7 (22.4-63.4)	69.3 (59.7-80.5)	61.3 (50.2-74.8)	61.9 (41.0-93.4)	47.4 (37.0-60.8)
90-94	75.8 (55.7-97.4)	N/A	N/A	120.6 (98-148.3)	N/A	57.4 (10.0-329.0)	58.2 (37.8-89.7)
Men							
Sample Size	2438	2279	5575	1058	717	1227	5698
Person-Years	9748	4533	11961	11934	4642	7896	60110
Dementia	191	95	173	224	95	140	492
Age Groups							
65-69	4.1 (1.5-7.2)	10.9 (5.7-20.6)	5.4 (2.6-11.3)	3.4 (1.5-7.3)	N/A	7.0 (4.1-12.0)	2.3 (1.2-4.4)
70-74	7.0 (4.9-9.3)	14.8 (8.0-27.5)	10.1 (5.8-17.6)	7.8 (5.1-11.7)	11.3 (0.3-396.8)	11.6 (6.2-21.7)	9.9 (6.9-14.2)
75-79	16.8 (13.3-20.6)	22.0 (8.1-59.2)	13.6 (7.0-26.6)	22.5 (17.5-28.9)	9.0 (2.3-34.8)	25.4 (16.2-39.8)	22.5 (16.7-30.3)
80-84	39.8 (33.1-46.4)	47.3 (27.6-81.2)	22.3 (12.4-38.9)	24.8 (18.6-33)	N/A	29.7 (15.9-55.6)	29.3 (20-42.9)
85-89	62.1 (45.1-76.2)	70.4 (35.8-138.3)	37.7 (22.4-63.4)	73.0 (56.5-94.3)	67.1 (45.1-100.0)	78.5 (27.9-220.6)	37.1 (20.1-68.3)
90-94	67.2 (36.2-100.2)	N/A	N/A	160.3 (111-231.7)	N/A	2.4 (0.2-33.4)	N/A
Women							
Sample Size	3326	4163	6914	1538	2307	1733	7958
Person-Years	14221	8317	14787	17972	24722	11735	88848
Dementia	286	166	300	461	517	297	1119
Age Groups							
65-69	4.7 (2.4-7.3)	6.4 (3.0-13.7)	4.7 (2.1-10.2)	1.7 (0.6-4.6)	7.1 (4.1-12.3)	8.8 (5.0-15.4)	2.7 (1.5-4.7)
70-74	8.5 (6.5-10.7)	7.9 (3.8-16.2)	6.1 (3.0-12.4)	9.2 (6.5-12.9)	8.3 (5.0-13.6)	16.8 (10.3-27.6)	8.7 (6.3-12)
75-79	14.9 (11.9-18.0)	17.5 (10.5-29.2)	16.8 (10.6-26.8)	17.8 (14.2-22.3)	22.2 (10.5-46.9)	36.9 (27.0-50.3)	19.9 (15.6-25.4)
80-84	35.8 (30.8-41.2)	37.6 (24.6-57.3)	39.3 (26.7-57.6)	40.9 (34.7-48.2)	38.9 (17.2-87.9)	47.6 (32.7-69.2)	29.2 (22.8-37.5)
85-89	68.8 (57.3-83.7)	50.6 (32.6-78.5)	45.8 (30.0-69.9)	67.7 (56.3-81.4)	59.5 (47.2-75.0)	61.5 (39.7-95.5)	50.2 (38.3-65.9)
90-94	79.9 (56.2-107.7)	N/A	N/A	108.6 (84.6-139.4)	N/A	95.0 (15.0-603.3)	74.1 (50.4-109)

Table 2. Age-specific incidence rates per cohort, overall and stratified by sex. N/A=not available because of insufficient numbers within category.

Several of the cohorts within the Alzheimer Cohorts Consortium have previously published data on time trends in the incidence of dementia.[4-7] The incidence trends described here are an important step towards consensus, as they add data from Scandinavia, corroborate the findings of individual studies using a consistent analytical techniques over a set calendar period, and affirm that these trends have benefitted men and women equally. The development we see now in dementia is somewhat reminiscent of the first report of a decline in mortality from coronary heart disease in 1964.[22] If history has thought us anything in that respect, it is the need for prolonged, consistent surveillance of disease and associated factors to enable modelling of trends and identification of causes.[22,23] As such, continued surveillance for dementia in the population-based studies within the Alzheimer Cohorts Consortium provides the framework for further investigation of potential causes of these trends, for which individual studies are generally underpowered.

The effect of a decline in age-specific incidence on the burden of disease needs to be seen against the backdrop of changes in life expectancy. Until the second half of the 20th century, the predominant view was that prolonged life expectancy would inescapably lead to higher burden of disease, but in 1980, James Fries proposed that this is not necessarily the case, as long as the factors accounting for prolonged survival are also linked with infirmity at old age.[24,25] This theory, designated the *compression of morbidity* would imply that longevity generally translates into a larger number of healthy life years, while the number of years lived with disease may remain stable or even decline. It is, however, uncertain whether this applies to cognitive morbidity and dementia.[26,27] Increases in life-expectancy in the UK between 1991 and 2011 seem paralleled by additional years spent with disability,[28] emphasising that further study is needed to determine the effect of declines in dementia incidence on the number of years lived with cognitive disability in an ageing population.[29]

The main challenge in pinpointing influences on time trends is that there have been many concurrent changes in addressing key risk factors that may have contributed to changes in incidence rates. While none of these have been specifically intended to halt cognitive decline, decades of cardiovascular risk management have likely had substantial effects on brain health, supported by a reduction of small-vessel disease on brain imaging in more recent years.[4] The challenge nevertheless remains to identify the 'culprit' among a variety of interventions influencing blood pressure, cholesterol, inflammation, platelet aggregation, and severity of heart disease and stroke, to name a few. Improved access and provision of education is another major change over the past century that has been marked as potential explanation for decreasing dementia incidence. Individuals with higher education are at lower risk of dementia, presumably because of so called *cognitive reserve,* defined as the ability of the brain to tolerate higher degrees of pathology before it falters to the level of

clinical dementia.[30] To understand the preventive potential of education, however, it is essential to determine whether education in fact modifies neurodevelopment, or that early brain development in utero – due to improvements in maternal health – influences cognitive trajectories and dementia outcomes in later life.

Contrasting reports have emerged recently from Japan,[31] China,[32] and Nigeria,[33] showing stable, or even increasing incidence of dementia. Against the backdrop of the large expected increases in dementia prevalence in particularly Asia and Africa,[21] these observations temper any optimism about disease burden, and render it all the more necessary to unravel the causes behind the trends seen in this study. Comparison with other geographical regions may well aid in pinpointing similar and discordant developments, and increased ethnic and geographic diversity within the Alzheimer Cohorts Consortium and the wider research community is therefore an important ongoing goal. This will require additional high quality surveillance data from understudied areas, and continuation of monitoring in ongoing studies alike. Similar to heart disease,[22] we should caution that the rise of obesity,[34] diabetes,[35] and (on a global level) hypertension,[36] may reverse trends in dementia over the coming decades.[37]

We have described methodological considerations for studying trends in the incidence of dementia previously.[9] First it is important to note that despite inevitable differences in population demographics, genetic and lifestyle make-up, and ascertainment methods for dementia, incidence rates and trends displayed only little heterogeneity across studies. Of other limitations, concurrent increases in life expectancy, and increased awareness of dementia in the population may have led to underestimation of a downward trend. Second, depletion of susceptible individuals in the closed cohort design of some of the included studies may have overestimated the decline in incidence, although trends were no different in those studies compared to studies with repeated sampling. Third, while the diagnostic criteria for dementia as a syndrome have remained rather constant over the past decades, insights in what should be called Alzheimer's disease have shifted substantially. In the absence of pathologically confirmed diagnoses, it therefore remains uncertain what pathological changes underlie the observed trends.

In conclusion, the incidence of dementia in Europe and North America has declined by 17% per decade of the past 25 years. If extended, this has substantial implications for the number of patients with dementia over the coming decades. Nevertheless, identification of the underlying causes is vital to prolong these trends in the face of changing risk factor profiles, and achieve equal reductions in other areas of the world where projected increases in dementia burden are steep, and improvements in incidence thus far absent.

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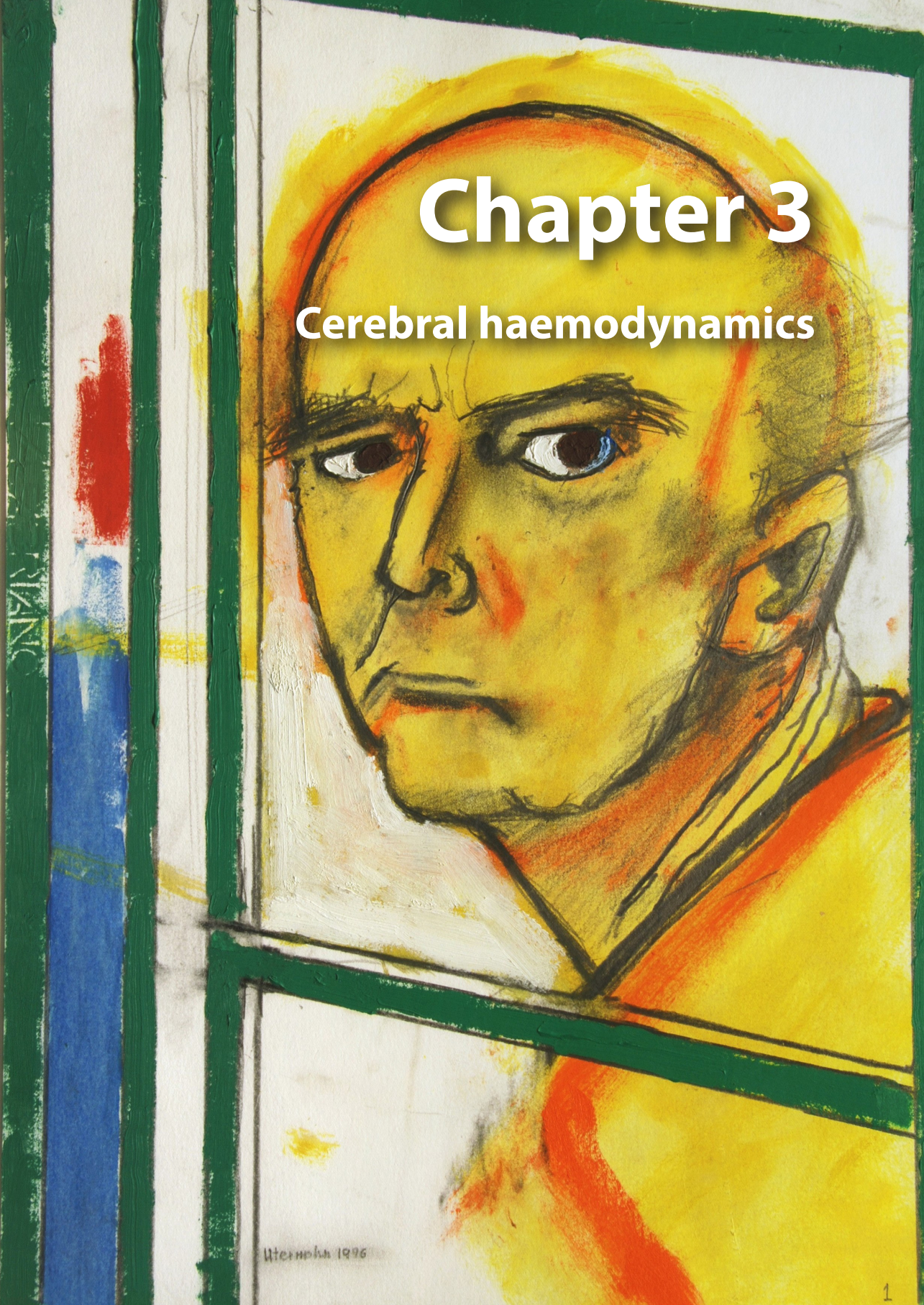

Chapter 3

Cerebral haemodynamics

Chapter 3.1

Cerebral perfusion

ABSTRACT

Various cross-sectional studies have reported lower cerebral perfusion in patients with mild cognitive impairment and dementia than in healthy controls, but the temporal relationship of these findings is under debate. Hypoperfusion may contribute to neurodegeneration by inducing neuronal energy crisis, but conversely loss of brain tissue can lead to reduced perfusion as metabolic demand decreases. We therefore prospectively determined the association of cerebral perfusion with subsequent cognitive decline and development of dementia. Between 2005 and 2012, we measured cerebral blood flow by 2D phase-contrast magnetic resonance imaging in non-demented participants of the population-based Rotterdam Study. We determined the association of cerebral perfusion (mL/100mL/minute) with risk of dementia (until 2015) using a Cox model, adjusting for age, sex, demographics, cardiovascular risk factors, and *APOE* genotype. We repeated analyses for Alzheimer's disease, and accounting for stroke. We furthermore determined change in cognitive performance during two consecutive examination rounds in relation to perfusion using linear regression, and investigated whether associations were modified by baseline severity of white matter hyperintensities (WMH). Of 4,759 participants (median age 61 years, 55% women) with a median follow-up of 6.9 years, 123 participants developed dementia (97 Alzheimer's disease). Lower cerebral perfusion was associated with higher risk of dementia (adjusted HR [95%CI] per standard deviation decrease: 1.31 [1.07-1.61]), similar for Alzheimer's disease only, and unaltered by accounting for stroke. Risk of dementia with hypoperfusion was higher with increasing severity of WMH (with severe WMH– HR 1.54 [1.11-2.14]). At cognitive re-examination after on average 5.7 years, lower baseline perfusion was associated with accelerated decline in cognition (global cognition: β=-0.029, *P*=0.003), which was similar after excluding those with incident dementia, and again most profound in individuals with higher volume of WMH (*P*-interaction=0.019). In conclusion, cerebral hypoperfusion is associated with accelerated cognitive decline and an increased risk of dementia in the general population.

INTRODUCTION

About 48 million people worldwide are living with dementia, and this number is predicted to increase to 131 million by 2050.[1] Consequently, the social and economic burden of dementia will increase enormously, unless preventive or curative measures can be established. Vascular disease is an important contributor to dementia, including Alzheimer's disease,[2,3] but the underlying pathophysiological mechanisms remain largely unknown. As vascular risk factors have an important effect on cerebral hemodynamics, cerebral hypoperfusion has been suggested as a potential link between vascular damage and dementia, and a potential target for preventive interventions.[4,5] Various cross-sectional studies have indeed reported lower perfusion in patients with mild cognitive impairment or dementia,[6-10] but the temporal relationship of these findings is debated.[11,12] Hypoperfusion may contribute to neurodegeneration by inducing neuronal energy crisis, while conversely loss of brain tissue can lead to hypoperfusion due to reduced metabolic demand. In fact, we recently found in a large longitudinal imaging study that smaller brain volume precedes decline in cerebral blood flow, whereas conversely low flow was associated with accelerated brain atrophy in elderly individuals.[13] Moreover, lower perfusion has been associated with more decline on the mini-mental state examination in the years preceding flow measurement,[14] but to date no studies have determined risk of developing dementia after a baseline measurement of cerebral blood flow.

Cerebral hypoperfusion has particularly been implicated in small-vessel disease, which is a major risk factor for dementia.[15,16] Hypoperfusion is suggested to play an important role in the pathophysiology of small vessel disease through ischemia and inflammation.[12,17] In addition, hypoperfusion may be particularly detrimental to neurons in the presence of capillary dysfunction or arteriolar disease, due to concomitant impaired vasoreactivity,[18] blood-brain barrier dysfunction,[19] and less efficient extraction of oxygen and other diffusible nutrients.[20] A cross-sectional study in patients with manifest arterial disease indeed found that hypoperfusion was particularly associated with worse executive function in the presence of more extensive white matter hyperintensities.[21] However, whether this also applies to other cognitive domains or to associations with subsequent cognitive decline and development of dementia is unknown.

In a prospective population-based cohort study, we aimed to determine the association of cerebral perfusion with subsequent cognitive decline and development of dementia, and to investigate whether this association varies with severity of small-vessel disease.

METHODS

Study population

This study is embedded within the Rotterdam Study, a large population-based cohort study in the Netherlands.[22] The original study population consisted of 7,983 participants aged ≥55 years from the Ommoord area, a suburb of Rotterdam. The cohort was subsequently expanded with 3,011 persons (≥55 years) in the year 2000, and an additional 3,932 persons (≥45 years) in 2005, thus including a total 14,926 participants in the cohort. From August 2005 onwards, all participants without contraindications are invited for magnetic resonance imaging (MRI). Contraindications are presence of iron-based metal implants, other internal metallic objects, severe claustrophobia, recent surgery, or the inability to lie flat for the duration of the scan. The current study includes all eligible participants, who underwent baseline MRI between 2005 and 2012 (N=5,163; 88.3% of invitees).

MRI scan protocol and image processing

MRI of the brain was performed on a 1.5 T scanner (General Electric Healthcare, Milwaukee, WI, USA), using an 8-channel head coil.[23] We acquired high-resolution axial T1-weighted sequence, proton-density-weighted (PD) sequence, and fluid attenuated inversion recovery (FLAIR) sequence. For flow measurement, 2D phase-contrast imaging was performed as described previously.[24] In brief, a sagittal 2D phase-contrast angiographic scout image was performed. On this scout image, a transverse imaging plane perpendicular to both the pre-cavernous portion of the internal carotid arteries and the middle part of the basilar artery was chosen for a 2D gradient-echo phase-contrast sequence (repetition time=20 ms, echo time=4 ms, field of view=19 cm2, matrix=256 × 160, flip angle=8°, number of excitations=8, bandwidth=22.73 kHz, velocity encoding=120 cm/sec, slice thickness=5 mm). Acquisition time was 51 seconds and no cardiac gating was performed.[25]

Flow was calculated from the phase-contrast images using interactive data language-based custom software (Cinetool version 4; General Electric Healthcare). Two experienced technicians drew all the manual regions of interest and performed subsequent flow measurements (inter-rater correlations >0.94 for all vessels).[24] This method for blood flow measurement was established in 1998,[26] and subsequent reports have demonstrated good accuracy and reproducibility.[24,25] Recently, phase contrast imaging has been shown to correlate well with arterial spin labelling (ASL) measures of cerebral perfusion,[27,28] although absolute estimates tend to be higher than with ASL and somewhat more variable.[27] For the assessment of brain volume, the structural MR sequences (T1-weighted, PD-weighted, and FLAIR) were transferred to a Linux workstation. Pre-processing steps and the classification algorithm have been described previously.[29] Quantification of cerebrospinal fluid,

parenchymal volume, and white matter hyperintensity (WHM) volume were done using an automated tissue segmentation method, based on a k-nearest-neighbor brain tissue classifier algorithm.[29] All segmentation results were visually inspected and if needed manually corrected. We calculated total brain perfusion (mL/min per 100 mL) by dividing total cerebral blood flow (mL/min) by each individual's brain volume (mL) and multiplying the result by 100. All scans were rated by trained research physicians, blinded to clinical data, for the presence of cerebral microbleeds (defined as small round to ovoid areas of focal signal loss on T2 susceptibility-weighted images), cortical infarcts, and lacunar infarcts (defined as focal lesions ≥3 and <15mm in size with similar signal intensity as cerebrospinal fluid and, when located supratentorially, a hyperintense rim on FLAIR).

Cognitive function assessment

Cognitive function was assessed in detail at baseline and follow-up with a neuropsychological test battery comprising the letter-digit substitution task (LDST, number of correct digits in 1 minute), the verbal fluency test (VF, animal categories), the Stroop test (error-adjusted time in seconds), a 15-word learning test (WLT, immediate and delayed recall), and Purdue pegboard task.[30] For each participant, Z-scores were calculated for each test separately, by dividing the difference between individual test score and mean test score by the standard deviation. We derived scores on cognitive domains for memory (WLT), information processing (Stroop reading and color naming task, and LDST (weighted half)), executive function (Stroop interference task, VF, and LDST (weighted half)), and motor function (Purdue pegboard test). To obtain a measure of global cognitive function, we furthermore calculated a standardized compound score (*g*-factor) using principal component analysis, including each of the cognitive tests described above.[30] The *g*-factor explained 47.4% of the variance in cognitive test scores in the population. The average interval between baseline assessment and re-examination was 5.7 years, limiting any practice effects.

Dementia screening and surveillance

Participants were screened for dementia at baseline and subsequent centre visits using the Mini-Mental State Examination (MMSE) and the Geriatric Mental State Schedule (GMS) organic level.[31] Those with MMSE<26 or GMS>0 underwent further investigation and informant interview including the Cambridge Examination for Mental Disorders of the Elderly. Additionally, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study centre with medical records from general practitioners and the regional institute for outpatient mental healthcare. Available clinical neuroimaging data were reviewed when required for diagnosis of dementia subtype. A consensus panel led by a consultant neurologist established the final diagnosis according to

standard criteria for dementia (DSM-III-R), and Alzheimer's disease (NINCDS-ADRDA). Follow-up until January 2015 was virtually complete (96.1% of potential person-years). Participants were censored within this follow-up period at date of dementia diagnosis, death, or last follow-up, whichever came first.

Other measurements

We assessed educational attainment (classified into lower, further, and higher education), civil status, residential situation (i.e. independent or with care), history of smoking (i.e. current, former, never) and use of antihypertensive or lipid-lowering medication at baseline by interview. Systolic and diastolic blood pressures were measured twice on the right arm with a random-zero sphygmomanometer; the mean of these readings was used for analyses. Mean arterial pressure was calculated by the sum of diastolic pressure and one-third times the difference between systolic and diastolic pressure. Fasting serum lipid levels were measured at baseline. Diabetes was defined as the use of blood glucose-lowering medication at baseline or a fasting serum glucose level ≥126 mg/dL. Body mass index was computed from measurements of height and weight (kg/m2). Carotid stenosis (≥50%) was assessed by Doppler ultrasound. History of stroke was assessed at baseline by interview and verified using medical records, and participants were continuously monitored for occurrence of incident stroke through computerized linkage of medical records from general practitioners and nursing home physicians with the study database. Ethnicity was determined from genotype. *APOE* genotype was determined by polymerase chain reaction on coded DNA samples in the original cohort, and by bi-allelic TaqMan assays (rs7412 and rs429358) for the expansion cohorts. In 177 participants with missing *APOE* status from this blood sampling, genotype was determined by genetic imputation (Illumina 610K and 660K chip; imputation with Haplotype Reference Consortium (HRC) reference panel (v1.0) with Minimac 3). Overall, *APOE* genotype was determined in 97.6% of participants, and classified into homozygote ε3 carriers, ε2 carriers (i.e. ε2/2 and ε2/3), and ε4 carriers (i.e. ε2/4, ε3/4 and ε4/4).

Analysis

Analyses included all non-demented participants who underwent MRI. Missing covariate data (maximum 10%) were imputed using 5-fold multiple imputation with an iterative Markov chain Monte Carlo method, based on determinant, outcome and included covariates. Distribution of covariates was similar in the imputed vs. non-imputed dataset.

We first determined the association between various cardiovascular risk factors and baseline cerebral perfusion by using linear regression. We then assessed change in cognitive test scores between examination rounds in relation to perfusion, using linear regression with test score at re-examination as the dependent variable, while adjusting for baseline test score,

age, age^2, sex, educational attainment, ethnicity, household income, smoking, mean arterial pressure, antihypertensive drugs, serum total cholesterol and high-density lipoprotein, lipid-lowering drugs, diabetes, body mass index, and *APOE* genotype. We repeated these analyses stratified by median age (61.3 years), and after exclusion of participants who were diagnosed with dementia prior to the repeated cognitive assessment. Finally, we assessed effect modification by WMH volume for global cognition and separate cognitive domains. To avoid overfitting of the models in the latter stratified analyses, adjustment for covariates other than baseline test score, age, and sex was done by means of propensity scores.

Next, we determined the association of cerebral perfusion with incident dementia, using Cox proportional hazard models. The proportional hazard assumption was met. We assessed risk of dementia per quartile of cerebral perfusion, as well as continuously per standard deviation (SD) decrease, thereby assessing for non-linearity with restricted cubic splines. All analyses were adjusted for age, age^2 and sex. We verified that age was sufficiently controlled for by comparing results with those from a model using cubic splines, and repeating the analyses with age rather than follow-up time as the time-scale. To minimize confounding by cardiovascular disease, in a second model we further adjusted for smoking history, mean arterial pressure, use of antihypertensive medication, serum total cholesterol and high-density lipoprotein, use of lipid-lowering medication, diabetes, body-mass index, and *APOE* genotype. In this model we furthermore controlled for ethnicity, educational attainment, civil status, and living condition. We repeated the analyses, 1) assessing Alzheimer's disease only, 2) excluding all participants with prior clinical stroke or MRI defined cortical infarct at baseline, while censoring for incident clinical stroke during follow-up, 3) with delayed entry after 1, 2, 3, and 4 years from baseline, and 4) excluding participants with carotid artery stenosis >50%. In addition, we examined potential mediation by small-vessel disease, by further adjusting for MRI markers of cerebral small vessel disease (i.e. WMH volume, cerebral microbleeds, and lacunar infarcts). Finally, we explored effect modification by age, sex, baseline levels of mean arterial pressure, and WMH volume at baseline, by stratifying analyses and testing for multiplicative interaction (entering perfusion and WMH volume as continuous variables in the model). Propensity scores were again used to avoid overfitting of the models in the stratified analyses. We visualized the association between perfusion and dementia by mean arterial pressure, creating 3D mesh plots (using negative exponential smoothing, 2nd degree polynomial, and nearest neighbor bandwidth method).

Analyses were done using SPSS Statistics version 23.0 (IBM Corp, Armonk, NY, USA), apart from analyses using splines and age as a time-scale for which we used R statistical software version 3.1.1 (packages 'rms' and 'survival'). 3D mesh plots were created using SigmaPlot version 8.0 (Systat Software, San Jose, CA). Alpha-level (type 1 error) was set at 0.05.

RESULTS

Of 5,010 eligible participants, no reliable measure of cerebral blood flow could be obtained in 58 (1.2%) persons, due to incorrect positioning of the phase-contrast imaging plane. In addition, parenchymal volume computations were unreliable in 193 (3.9%) participants, due to inadequate quality of obtained images, thus leaving a total of 4,759 (95.0%) individuals for analysis. Baseline characteristics of participants are presented in Table 1.

Characteristics	**Study population** (*N*=4,759)	**With cognitive re-examination** (*N*=3,700)	**No cognitive re-examination** (*N*=1,059)
Age, years	63.7 (±10.8)	62.2 (±9.7)	69.1 (±12.8)
Female sex	2625 (55.2%)	2031 (54.9%)	565 (56.1%)
Caucasian ethnicity	4156 (97.3%)	3219 (97.0%)	891 (98.5%)
Smoking			
Former	2300 (48.6%)	1807 (49.1%)	474 (47.4%)
Current	995 (21.0%)	731 (19.9%)	249 (24.9%)
Systolic blood pressure, mmHg	139 (±21)	138 (±20)	143 (±23)
Diastolic blood pressure, mmHg	82 (±11)	82 (±11)	82 (±12)
Mean arterial pressure, mmHg	101 (±13)	101 (±13)	102 (±14)
Antihypertensive medication	1616 (34.2%)	1130 (30.8%)	462 (46.2%)
Cholesterol, mg/dL	215 (±41)	216 (±41)	211 (±41)
HDL cholesterol, mg/dL	56 (±16)	56 (±16)	54 (±15)
Lipid lowering medication	1129 (23.9%)	848 (23.1%)	267 (26.7%)
Diabetes	519 (11.1%)	368 (10.1%)	146 (14.9%)
Body-mass index, kg/m^2	27.4 (±4.2)	27.5 (±4.1)	27.4 (±4.4)
Educational attainment			
Lower	2180 (46.2%)	1621 (44.2%)	531 (53.1%)
Further	1440 (30.5%)	1129 (30.8%)	295 (29.5%)
Higher	1100 (23.3%)	918 (25.0%)	174 (17.4%)
Civil status			
Living with spouse or partner	3540 (74.8%)	2870 (77.9%)	636 (63.7%)
Widowed, divorced, or never married	1191 (25.2%)	813 (22.1%)	363 (36.3%)
Residential care	270 (5.7%)	155 (4.2%)	110 (11.0%)
APOE genotype			
ε3/ε3	2726 (58.7%)	2127 (58.8%)	574 (58.8%)
ε2/ε2 or ε2/ε3	604 (13.0%)	472 (13.0%)	122 (12.5%)
ε2/ε4, ε3/ε4, or ε4/ε4	1315 (28.3%)	1020 (28.2%)	281 (28.8%)
Carotid artery stenosis (≥50%)	208 (4.4%)	112 (3.1%)	91 (9.2%)
Cerebral perfusion, mL/100mL/min	56.3 (±9.7)	56.7 (±9.5)	54.9 (±10.1)

Table 1. Baseline characteristics. Values are depicted as mean±SD for continuous variables, and absolute numbers (%) for categorical variables. N=sample size; *APOE*=apolipoprotein E; SD=standard deviation

Cerebral perfusion was lower with advancing age, and lower in men compared with women (Table 2). Most cardiovascular risk factors were individually associated with perfusion at baseline, whereas after adjustment for other risk factors associations with use of antihypertensive medication, cholesterol level, and current smoking remained statistically significant (Table 2).

Of 4,707 participants (98.9%) who underwent detailed cognitive assessment at baseline, 3,700 (78.6%) had repeated assessment at follow-up (mean interval 5.7 years). Lower cerebral perfusion at baseline was associated with accelerated decline in global cognition, particularly in memory and executive function (Table 3). Across domains, effect estimates for perfusion increased with increasing severity of white matter hyperintensities (WMH) (*P*-value for interaction of perfusion and WMH with respect to global cognition=0.019; Figure 1). Associations were also stronger in older compared with younger participants (*P*-value for interaction=0.018; Table 3). Results were similar when excluding participants who were diagnosed with dementia prior to cognitive re-assessment (β [95% CI] for global cognition: -0.029 [-0.048;-0.010]).

Determinant	Effect on cerebral perfusion	
	Model I (β, 95% CI)	Model II (β, 95% CI)
Age, per 10 years	-0.217 (-0.243;-0.192)	-0.189 (-0.216;-0.162)
Female sex	0.425 (0.371;0.479)	0.419 (0.358;0.479)
Hypertension‡	-0.104 (-0.161;-0.047)	n/a
Mean arterial pressure, per 10mmHg	-0.015 (-0.037;0.006)	0.001 (-0.021;0.023)
Systolic blood pressure, per 10mmHg	-0.013 (-0.028;0.001)	n/a
Diastolic blood pressure, per 10mmHg	-0.010 (-0.035;0.015)	n/a
Anti-hypertensive medication	-0.151 (-0.211;-0.092)	-0.120 (-0.185;-0.055)
Smoking		
Never	REFERENCE	REFERENCE
Former	-0.058 (-0.122;0.006)	-0.053 (-0.117;0.010)
Current	0.129 (0.051;0.207)	0.132 (0.053;0.210)
Total cholesterol, per 1mmol/L	-0.019 (-0.045;0.008)	-0.044 (-0.072;-0.015)
HDL cholesterol, per 1mmol/L	0.088 (0.017;0.158)	0.068 (-0.008;0.144)
Lipid-lowering medication	-0.083 (-0.147;-0.019)	-0.061 (-0.130;0.009)
Diabetes	-0.092 (-0.180;-0.004)	-0.053 (-0.145;0.038)
Body mass index, per 5 points	-0.061 (-0.094;-0.028)	-0.025 (-0.062;0.012)

Table 2. Determinants of cerebral perfusion. Model I is adjusted for age and sex, if applicable, whereas all presented variables are included in model II. ‡ blood pressure ≥160/90 or use of anti-hypertensive medication CI=confidence interval; n/a=not applicable. Values reflect standardised regression coefficient with 95% CI.

	All participants β for change (95% CI)	Age <61 years β for change (95% CI)	Age ≥61 years β for change (95% CI)
Global cognition	-0.029 (-0.048;-0.010)	-0.010 (-0.032;0.013)	-0.056 (-0.089;-0.022)
Memory	-0.031 (-0.056;-0.006)	-0.013 (-0.045;0.019)	-0.047 (-0.086;-0.008)
Information processing	-0.007 (-0.024;0.009)	0.003 (-0.017;0.023)	-0.020 (-0.047;0.007)
Executive function	-0.017 (-0.033;-0.001)	-0.013 (-0.032;0.007)	-0.025 (-0.052;0.002)
Motor function	-0.001 (-0.026;0.027)	0.014 (-0.026;0.055)	-0.025 (-0.051;0.001)

Table 3. Cerebral perfusion and change in cognitive test performance. Betas represent the effect of cerebral perfusion per SD decrease on standardized cognitive test score at follow-up examination, adjusted for baseline cognitive test score. Results are stratified by the median age of 61.3 years. The model is adjusted for age, sex, educational attainment, ethnicity, civil status, residential care, smoking, mean arterial pressure, antihypertensive drugs, serum total cholesterol and high-density lipoprotein, lipid-lowering drugs, diabetes, body mass index, and *APOE* genotype. CI=confidence interval; SD=standard deviation.

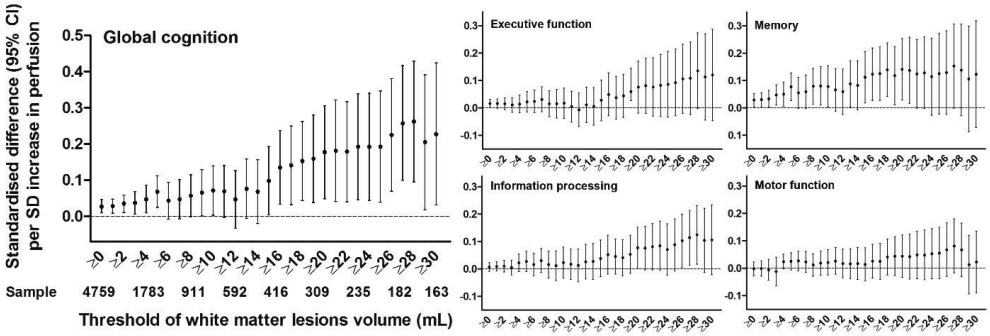

Figure 1. Cerebral perfusion and change in cognitive performance by severity of white matter hyperintensities. Results are presented for global cognition and each of the separate cognitive domains. Moving right along the x-axis limits the included population to those with at least the specified volume of white matter hyperintensities on baseline MRI (ranging from the full sample of 4,759 individuals with ≥0mL to a sample of 163 individuals with ≥30mL). Each dot represents the estimated change in cognitive test performance per 1 standard deviation increase in perfusion in this specified population. CI=confidence interval

During a mean follow-up time of 6.9 years, 123 individuals developed dementia, of whom 97 (78.9%) had Alzheimer's disease. Follow-up for dementia was virtually complete for all 4759 participants (96.1% of potential person years). Of incident dementia cases, 25 were preceded by a clinical stroke or had evidence of cortical infarction on baseline MRI.

Lower cerebral perfusion at baseline was associated with a higher risk of dementia (adjusted HR [95% CI] per SD decrease: 1.31 [1.07-1.61]), with similar effect estimates for Alzheimer's disease (Table 4). There was no evidence of non-linearity in the association between perfusion and dementia. Results were unaffected by excluding prevalent stroke and censoring at time of incident stroke (HR 1.33, 1.06-1.68). Analyses with delayed study entry, excluding the first year of follow-up resulted in mildly reduced estimates, which remained grossly stable with additional exclusion of the 2nd, 3rd, and 4th year of follow-up (HRs 1.26, 1.24, 1.21, and 1.25, respectively). Overall effect estimates were mildly attenuated after excluding participants with ≥50% carotid artery stenosis (HR 1.23 [0.99-1.53]), and when adjusting for MRI markers of small vessel disease (HR 1.25 [1.02-1.54]; Table 5).

The association between cerebral perfusion and risk of dementia was more profound with increasing burden of WMH on MRI (Table 5 – with severe WMH: HR 1.54 [1.11-2.14]), although a formal test for multiplicative interaction was not statistically significant (*P*=0.24). This trend was unaltered by excluding all 222 participants with prior stroke or infarcts on MRI at baseline. In addition, dementia risk estimates for low perfusion were higher in those with higher blood pressure levels at baseline (Figure 2 – *P*-value for interaction with mean arterial pressure = 0.039). This trend was consistently seen for systolic and diastolic pressure

(Figure 3A), and persisted after additional adjustment for WMH volume. There was no effect modification of the association between cerebral perfusion and dementia risk by age or sex.

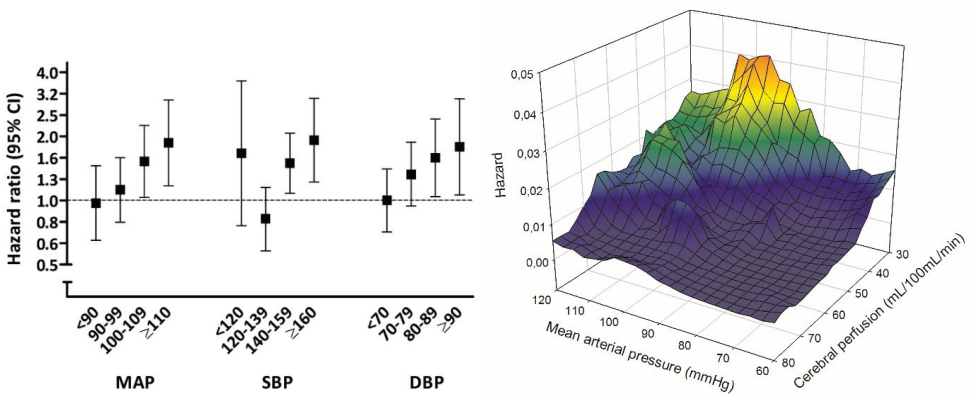

Figure 2. Cerebral perfusion and dementia risk by baseline blood pressure levels (A), and 3D-graphically depicted for mean arterial pressure (B). MAP=mean arterial pressure; SBP=systolic blood pressure; DBP=diastolic blood pressure; CI=confidence interval

DISCUSSION

In this large population-based study, we found that lower cerebral perfusion at baseline was associated with accelerated cognitive decline and higher risk of developing dementia during on average 7 years of follow-up. These associations were most profound in individuals with higher volume of white matter hyperintensities (WMH) or higher mean arterial pressure.

Prior studies have almost invariably shown associations of hypoperfusion with mild cognitive impairment and Alzheimer's disease in cross-sectional studies,[6-9] and more rapid decline in cognition after diagnosis of dementia in a longitudinal study.[10] Lower perfusion is often attributed to neurodegeneration, and can indicate neuronal dysfunction and synaptic failure. The first signs of neurodegeneration are likely to occur years prior to diagnosis of dementia, and cerebral perfusion may consequently fall well before clinical symptoms of dementia arise. Nevertheless, our findings show that the association of perfusion with cognitive decline extends well into the pre-symptomatic phase of the disease, and could therefore precede and contribute to neuronal cell loss and neurodegeneration also. Both sides of this medal are supported by a recent longitudinal imaging study, in which smaller brain volume not only precipitated decline in cerebral blood flow, but low flow also predisposed to accelerated brain atrophy in elderly individuals.[13] In line with these findings, we found strongest associations of hypoperfusion with cognitive decline in those over 60 years of age, which extended to individuals who did not (yet) develop dementia.

	All-cause dementia			Alzheimer's disease		
	N_{dem}/N_{total}	Model I HR (95% CI)	Model II HR (95% CI)	N_{dem}/N_{total}	Model I HR (95% CI)	Model II HR (95% CI)
Quartiles of perfusion						
Q1 $_{<50\ mL/100mL/min}$	51/1189	2.28 (1.20-4.32)	2.27 (1.19-4.32)	41/1189	2.15 (1.06-4.33)	2.08 (1.02-4.26)
Q2 $_{50-55\ mL/100mL/min}$	40/1190	2.35 (1.23-4.49)	1.95 (1.01-3.77)	31/1190	2.13 (1.04-4.37)	1.63 (0.78-3.41)
Q3 $_{56-62\ mL/100mL/min}$	20/1190	1.27 (0.62-2.61)	1.20 (0.59-2.47)	15/1190	1.13 (0.51-2.51)	1.03 (0.46-2.30)
Q4 $_{>62\ mL/100mL/min}$	12/1190	REFERENCE	REFERENCE	10/1190	REFERENCE	REFERENCE
P-trend		0.002	0.002		0.007	0.008
Per SD decrease	123/4759	1.30 (1.07-1.58)	1.31 (1.07-1.61)	97/4759	1.26 (1.01-1.57)	1.28 (1.01-1.62)

Table 4. Cerebral perfusion and risk of dementia. Model I is adjusted for age and sex, and model II additionally for educational attainment, ethnicity, civil status, residential care, smoking, mean arterial pressure, antihypertensive drugs, total cholesterol and HDL cholesterol, lipid-lowering drugs, diabetes, body mass index, and *APOE* genotype. HR=hazard ratio; CI=confidence interval; SD=standard deviation

	Adjustment for small-vessel disease*		By severity of white matter hyperintensities†		
	N_{dem}/N_{total}	HR (95% CI)	None to mild (N_{dem}/N_{tot}=40/3439)	Moderate (N_{dem}/N_{tot}=40/763)	Severe (N_{dem}/N_{tot}=39/443)
Quartiles of perfusion					
Q1	50/1166	2.08 (1.08-4.00)	1.18 (0.43-3.23)	3.14 (0.91-10.77)	4.36 (1.29-14.72)
Q2	37/1164	1.94 (1.00-3.77)	1.26 (0.45-3.53)	2.60 (0.73-9.35)	1.96 (0.51-7.58)
Q3	20/1160	1.19 (0.58-2.45)	1.71 (0.67-4.38)	2.45 (0.65-9.32)	1.66 (0.42-6.68)
Q4	12/1155	REFERENCE	REFERENCE	REFERENCE	REFERENCE
P-trend		0.007	0.98	0.083	0.003
Per SD decrease	119/4645	1.25 (1.02-1.54)	1.07 (0.76-1.51)	1.30 (0.93-1.84)	1.54 (1.11-2.14)

Table 5. Cerebral perfusion and dementia in the context of cerebral small-vessel disease. * Adjusted for age, sex, educational attainment, ethnicity, civil status, residential care, smoking, mean arterial pressure, antihypertensive drugs, total cholesterol and HDL cholesterol, lipid-lowering drugs, diabetes, body mass index, *APOE* genotype, volume of white matter hyperintensities, presence of lacunar infarcts, and cerebral microbleeds. † Adjusted by means of propensity score. Categories based on an approximately equal number of cases across categories (cut-offs at 6mL and 15mL, respectively). HR=hazard ratio; CI=confidence interval.

Various potential underlying mechanisms can link hypoxia to (neuronal) cell death, many of which are related to activation of hypoxia-inducible transcription factors (HIF). HIF can lead to increased expression of various inflammatory cytokines,[32] and the subsequent activation of microglia,[33] release of pro-inflammatory neurotoxic factors, and oxidative stress may explain part of the observed link between neuro-inflammation and Alzheimer's disease.[34] HIF furthermore renders endothelial cells responsive to various proangiogenic factors, as seen in the white matter of patients with Alzheimer's disease.[35] These proangiogenic factors are important for maintaining blood-brain barrier integrity through regulating endothelial cell and pericyte function in angiogenesis,[36] and dysfunction of these vital components of the neurovascular unit has been implicated in neurodegeneration with Alzheimer's disease.[36] Moreover, hypoxia can result in aberrant angiogenesis and microvascular degeneration in humans via pathways that are associated with advanced vascular degeneration and poor β-amyloid clearance in mice.[37] Cerebral blood flow correlates with amyloid burden across the spectrum from cognitively healthy to Alzheimer's disease,[38] which could be in part consequential, and in part contributing to impaired amyloid clearance. Certain areas in the brain, such as the metabolically highly active hippocampi, may be particularly vulnerable to hypoxia, which could explain their role in early Alzheimer's disease,[39] and the marked associations we found with memory function in our study. Future studies may focus more specifically on such regions, refine insight in these pathways, and investigate whether cerebral perfusion or hypoxia mediates associations of for instance heart failure and atrial fibrillation with dementia.

Hypoperfusion is widely implicated in the etiology of cerebral small-vessel disease, but once again the temporality of the association is under debate.[12,17,40] The mild attenuation of risk estimates by adjusting for markers of cerebral small-vessel disease in our study may in that respect reflect confounding or partial mediation of the association between hypoperfusion and dementia by small-vessel disease. In addition, small-vessel disease may modify an effect of hypoperfusion on neuronal cell loss. In line with a prior cross-sectional study of executive functioning,[21] we observed stronger associations in individuals with higher degree of WMH at baseline. WMH have been related to blood brain barrier permeability,[41] diminished vasoreactivity,[42] and a state of impaired extraction of oxygen and other nutrients, in which hypoperfusion could be especially hazardous to meeting metabolic demand.[20] Diminished blood-brain barrier function may render amyloid clearance more dependent on interstitial bulk flow,[43] while in mouse models of Alzheimer's disease, vascular dysfunction and hypoperfusion lead to impaired drainage of interstitial fluid and β-amyloid clearance.[44] Of particular relevance to brain tissue, encased as it is by the skull, is its low interstitial compliance, causing small increases in interstitial volume to lead to large increases in interstitial pressure. Consequently, increases in arterial pressure may be required to

maintain the hydrostatic pressure gradient and fluid filtration. This might underlie the observed interaction between perfusion and arterial blood pressure in our study. Yet, high blood pressures may also reflect longstanding hypertension and its detrimental consequences on (micro)vascular integrity and function.[45] The potential interplay between blood pressure, arteriolar and capillary dysfunction, and neuronal hypoxia warrants further investigation. Of note, somewhat counterintuitively, *hyper*perfusion might also lead to lower oxygen extraction in the presence of relatively mild-moderate capillary dysfunction, requiring suppression of blood flow to optimize metabolism.[46] In those individuals, perfusion may be reduced as a mechanism to optimize oxygen extraction. Repeated scan data in future studies may aid to further explore this possibility.

Although we believe our findings are valid, there are certain limitations to our study to take into account. First, 2D phase contrast flow measurement does not allow region specific assessment of cerebral perfusion, which is likely more sensitive in detecting associations with cognitive decline. Also, we could not differentiate between grey and white matter perfusion. Although phase contrast imaging measures of perfusion correlate well with ASL,[27,28] absolute estimates tend to be higher and somewhat more variable.[27] Such a systematic deviation would however not influence obtained relative risks, and a larger variability would only lead to dilution of effect estimates. Second, we could not measure cerebellar blood flow, as flow in the basilar artery was measured distally of the posterior and anterior inferior cerebellar arteries. Third, although follow-up for dementia was near-complete (96%), attrition for cognitive re-examination was substantial (21%). As those lost to follow-up were older, had worse risk profiles, and lower cerebral perfusion, this most likely led to an underestimation of the association of perfusion with decline in test performance. Response rate to MRI invitation in our study was 88.3%, and non-participants were also older than those who did undergo brain imaging. Fourth, given the long pre-symptomatic phase of dementia the median 7 years of follow-up is still relatively short, and we therefore cannot completely rule out reverse causation. Finally, the vast majority of our population is of European ancestry, potentially limiting generalizability to other ethnicities.

In conclusion, cerebral hypoperfusion is associated with accelerated cognitive decline and increased risk of dementia in the general population. These findings support a role of cerebral hypoperfusion in the pathophysiology of dementia. Further studies are warranted to unravel mechanisms in relation to blood pressure and small vessel disease, and assess the potential of cerebral perfusion as a target for prevention of cognitive decline.

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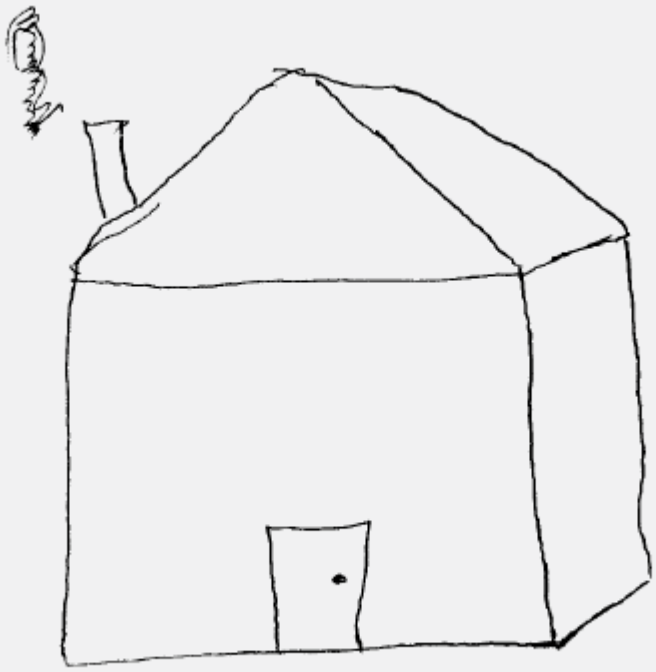
Chapter 3.2

Orthostatic hypotension



ABSTRACT

Orthostatic hypotension is a common cause of transient cerebral hypoperfusion in the population. Hypoperfusion and hypoxia are implicated in the pathophysiology of cognitive decline, but whether orthostatic hypotension predisposes to dementia is uncertain. Between 1990 and 1993, we assessed orthostatic hypotension in 6,204 non-demented, stroke-free participants of the population-based Rotterdam Study (mean age 69 years, 60% female). Orthostatic hypotension was defined as a ≥20mmHg drop in systolic or ≥10mmHg drop in diastolic blood pressure within 3 minutes from postural change. We furthermore calculated within subject variability in systolic blood pressure (SBP) related to postural change, expressed as the coefficient of variation (CV). We determined the risk of dementia (until 2014) in relation to orthostatic hypotension and SBP variability, using a Cox regression model, adjusted for age, sex, cardiovascular risk factors, relevant medication, and apolipoprotein E genotype. Finally, we explored whether associations varied according to the compensatory rise in heart rate. During a median follow-up of 15.3 years, 1176 participants developed dementia, of whom 935 (79.5%) had Alzheimer's disease and 95 (8.1%) vascular dementia. Orthostatic hypotension was associated with an increased risk of dementia (HR [95% confidence interval]: 1.15 [1.00-1.34]), comparable for Alzheimer's disease and vascular dementia. Similarly, greater SBP variability with postural change was associated with an increased risk of dementia (HR [95% CI] per standard deviation increase: 1.08 [1.01-1.16]), extending to participants who did not meet the formal criteria for orthostatic hypotension (HR 1.08 [1.00-1.17]). The risk of dementia was particularly increased in those with orthostatic hypotension who lacked compensatory increase in heart rate (within lowest quartile of heart rate response: HR 1.39 [1.04-1.85]; *P*-interaction=0.05). In conclusion, orthostatic blood pressure drops are associated with an increase in the long-term risk of dementia in the general population.

INTRODUCTION

Cardiovascular health is now well-established as a key determinant in the prevention of dementia, including Alzheimer's disease,[1,2] but the mechanisms by which vascular damage leads to cognitive decline remain largely unknown. As cerebral hypoperfusion is widely implicated in dementia,[3,4] cerebral haemodynamics have been suggested as a potential link between vascular risk factors and dementia.[5] Two important mechanisms for maintenance of proper and continuous cerebral perfusion are local vasoreactivity and autonomous nervous system function. Cerebral vasoreactivity has indeed been associated with the risk of developing dementia in the general population,[6] but the role of autonomous nervous system function in the onset of dementia has been less well-studied.

Autonomic dysfunction may result in orthostatic hypotension, which affects 20-30% of the elderly population.[7,8] Orthostatic hypotension is characterised by a marked drop in blood pressure following postural change, insufficiently compensated for by sympathetic and parasympathetic mechanisms. This may elicit transient (cerebral) hypoperfusion, especially in the absence of compensatory increase in heart rate. Orthostatic hypotension is associated with an increased risk of cardiovascular events, stroke, and mortality.[9] Moreover, orthostatic hypotension is highly prevalent among patients with dementia and mild cognitive impairment, compared to healthy controls,[10-13] but only one study assessed the longitudinal relation between orthostatic hypotension and the risk of dementia in initially healthy participants. In this Swedish population, orthostatic hypotension was associated with an increased risk of having dementia at re-examination after 6 years, but the investigators were unable to adjust for (cardiovascular) risk factors aside hypertension, and attrition was substantial with 37.5% of participants lost to follow-up between examination rounds.[14] These limited data regarding orthostatic hypotension and cognition prompted a recent review and meta-analysis to conclude that longitudinal studies using standardised criteria are needed to elucidate whether orthostatic hypotension is an independent risk factor for developing dementia.[9,15] We therefore aimed to determine the association between orthostatic hypotension and the risk of dementia in a long-term population-based study.

METHODS

Study population

This study is embedded within the Rotterdam Study, a large ongoing population-based cohort study in the Netherlands, with an initial study population of 7983 participants (78% of invitees) aged ≥55 years from the Ommoord area, a suburb of Rotterdam. The Rotterdam

Study methods have been described in detail previously.[16] In brief, participants were interviewed at home and examined at the research centre for baseline assessment from 1990 to 1993. Until 2015, five follow-up examinations have been carried out. Orthostatic hypotension was determined during baseline assessment. Of 7,983 participants, 7,157 (89.7%) visited the research centre for physical examination.

Assessment of orthostatic blood pressure change

Blood pressure and heart rate were measured using an automatic recorder (Dinamap R, Tampa, FL). The baseline blood pressure reading was the mean of two measurements on the right upper arm with the subject in supine position after 5 minutes of rest. Measurements were repeated in the standing position after 1, 2, and 3 minutes. Orthostatic hypotension was defined as ≥20mmHg decrease in systolic blood pressure or ≥10mmHg decrease in diastolic blood pressure after postural change at any of the three measurements, in accordance with the Consensus Committee of the American Autonomic Society and the American Academy of Neurology.[17,18] We defined severity of orthostatic hypotension by degree of blood pressure drop, i.e. ≥20/10 but <30/15, ≥30/15 but <40/20, and ≥40/20 mmHg. We calculated continuous measures of blood pressure change in response to postural change, expressed as the coefficient of variation of within subject variability (CV), defined as the ratio of the standard deviation to the mean of all measurements (i.e. measurements in supine and upright position combined). Furthermore, we determined the maximum increase in heart rate within 3 minutes after postural change, and asked participants directly afterwards whether they had felt unwell following postural change.

Dementia screening and surveillance

Participants were screened for dementia at baseline and subsequent centre visits using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level.[19] Those with MMSE<26 or GMS>0 underwent further investigation and informant interview including the Cambridge Examination for Mental Disorders of the Elderly. Additionally, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study centre with medical records from general practitioners and the regional institute for outpatient mental healthcare. Available clinical neuroimaging data were reviewed when required for diagnosis of dementia subtype. A consensus panel headed by a consultant neurologist established the final diagnosis according to standard criteria for dementia (DSM-III-R), and Alzheimer's disease (NINCDS-ADRDA), and vascular dementia (NINDS-AIREN). Follow-up until 1st January 2014 was near-complete (94.0% of potential person years), and participants were censored within this follow-up period at date of dementia diagnosis, death, loss to follow-up, or 1st January 2014, whichever came first.

Other measurements

We assessed smoking habits (i.e. current, former, never), alcohol intake, and baseline use of antihypertensive or anticholinergic medication by interview. Anti-cholinergic medication included anti-psychotic and anti-depressant medication, but also drugs prescribed against parkinsonism, urinary incontinence, or obstructive pulmonary disease that can have anticholinergic side-effects. Fasting serum lipid levels were measured at baseline. Hypertension was defined as the use of antihypertensive medication and/or elevated systolic or diastolic blood pressure (>140/90 mmHg). Body mass index was computed from measurements of height and weight (kg/m^2). Diabetes was defined as the use of blood glucose-lowering medication at baseline or a random serum glucose level ≥11.1 mmol/L.[20] Myocardial infarction and atrial fibrillation were assessed by interview and presence of abnormalities on a 12-lead electrocardiogram. Heart failure was determined using a validated score, similar to the definition of the European Society of Cardiology.[21] *APOE* genotype was determined using polymerase chain reaction on coded DNA samples.

Analysis

Analyses included all non-demented, stroke-free participants attending the study centre for examination. Of 7,157 participants attending the study centre, 531 were ineligible due to prevalent dementia (n=312), stroke (n=168), or both (n=51). Missing covariate data (maximum 17.6%), excluding *APOE* genotype, were imputed using fivefold multiple imputation, based on determinant (presence of orthostatic hypotension and postural systolic blood pressure variability), outcome and included covariates. Distribution of covariates was similar in the imputed versus non-imputed dataset.

We determined the association between presence of orthostatic hypotension and incident dementia, using Cox proportional hazard models. We repeated the analysis with dementia and death as the joint outcome measure, to reduce selection due to competing risk. Subsequently, we analysed categories of increasing severity of orthostatic blood pressure drops, and orthostatic hypotension with and without feeling unwell. Because of right-skewedness, we performed a natural logarithmic transformation of systolic blood pressure variability to obtain a roughly normal distribution (mean -2.52, standard deviation 0.58). Z-scores were computed by dividing the difference between the individual value and the population mean by the population standard deviation. We determined the association between blood pressure variability related to postural change per quartile and continuously per standard deviation increase, using a Cox model. To eliminate a paradoxical impact of high blood pressure variability in those with excessive increases, we repeated analyses after excluding those with ≥20mmHg systolic or ≥10mmHg diastolic increase in blood pressure within 3 minutes. Furthermore, we determined whether associations extended to those

without a formal diagnosis of orthostatic hypotension. We then assessed whether the risk of dementia in relation to orthostatic blood pressure drops was modified by response in heart rate after postural change, by testing for multiplicative interaction in the above Cox model and providing risk estimates of orthostatic hypotension for dementia per quartile of response in heart rate. We verified that the proportional hazard assumption was not violated in these models by plotting the partial (Schoenfeld) residuals against follow-up time. All analyses were adjusted for age and sex, and additionally in a second model for smoking habits, alcohol intake, systolic and diastolic blood pressure, use of antihypertensive medication, ratio of serum total cholesterol to HDL cholesterol, use of lipid-lowering medication, diabetes, body mass index, anti-cholinergic medication, and *APOE* genotype.

We repeated the analyses for Alzheimer's disease and vascular dementia separately, after censoring participants at time of incident stroke, after excluding those with Parkinson's disease at baseline, after excluding those with heart disease (i.e. coronary heart disease, heart failure, atrial fibrillation), and after excluding those with possible postural tachycardia syndrome (defined as a ≥30 beats per minute increase in heart rate, or any heart rate of ≥120 beats per minute). Finally, we performed several sensitivity analyses: 1) for men and women separately, 2) for persons above and below the median age (68.5 years), 3) excluding the first 5 years of follow-up to assess for reverse causality, 4) for those with and without heart failure at baseline, 5) for those with and without a history of hypertension, 6) distinguishing use of anti-hypertensive drugs, and 7) for those with and without diabetes.

All analyses were done using IBM SPSS Statistics version 23.0 (IBM Corp, Armonk, NY, USA). Alpha (type 1 error) was set at 0.05.

RESULTS

Of 6,626 eligible participants, 6,303 (95.1%) underwent examination for orthostatic hypotension. No baseline blood pressure measurement was obtained in 8 individuals, and no measurement after postural change in 91 individuals, leaving a total of 6,204 (93.6%) cases for analysis. Baseline characteristics of participants are shown in Table 1.

Overall, 1,152/6,204 (18.6%) participants had orthostatic hypotension. The prevalence of orthostatic hypotension steeply increased with age, to 30.6% of those aged >75 years. Although prevalence in the elderly was similar for men and women, there was a slightly higher prevalence in women at younger ages (Figure 1). Of all patients with orthostatic hypotension, 160 (13.9%) reported feeling unwell along with their blood pressure drop.

Characteristics	Study population
Age	68.5 ±8.6
Female sex	3704 (59.7)
Systolic blood pressure (mmHg)	139 ±22
Diastolic blood pressure (mmHg)	74 ±11
Antihypertensive medication	1901 (30.7)
Diabetes	421 (7.2)
Body-mass index (kg/m^2)	26.3 ±3.6
Total cholesterol (mmol/L)	6.6 ±1.2
HDL cholesterol (mmol/L)	1.4 ±0.4
Lipid-lowering medication	150 (2.4)
Smoking	
Former	2495 (41.9)
Current	1257 (21.1)
Alcohol intake (grams/day, median, IQR)	3.4 (0.2-14.8)
Anti-cholinergic medication	1391 (22.4)
APOE genotype	
ε3/ε3	3457 (58.3)
ε2/ε2, ε2/ε3, or ε2/ε4	978 (16.4)
ε3/ε4 or ε4/ε4	1494 (25.3)
Orthostatic hypotension	1152 (18.6)
≥20/10 mmHg, but <30/15 mmHg	773 (12.5)
≥30/15 mmHg, but <40/20 mmHg	239 (3.9)
≥40/20 mmHg	140 (2.3)
Blood pressure variability* (median, IQR)	0.08 (0.06-0.12)

Table 1. Baseline characteristics of the 6,204 study participants. Non-imputed data presented as frequency (%) for categorical, and mean±standard deviation for continuous variables, unless indicated otherwise; IQR=interquartile range; *expressed as coefficient of variation.

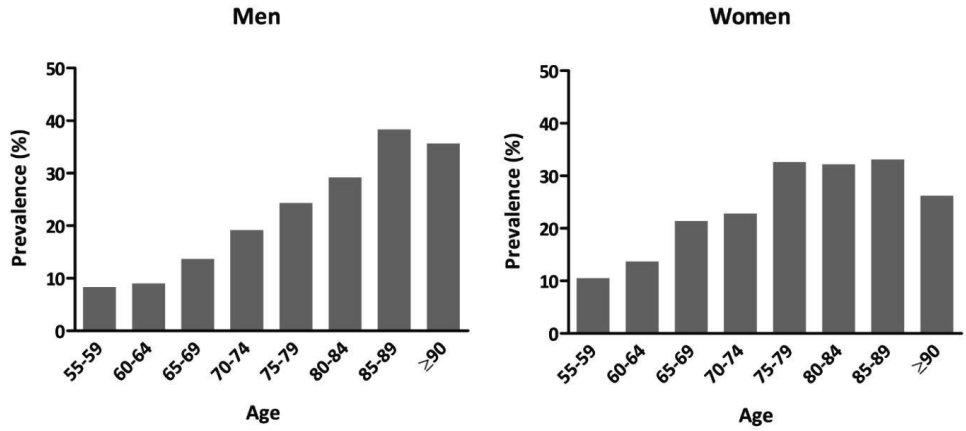


Figure 1. Age-specific prevalence of orthostatic hypotension in men and women.

During a median follow-up time of 15.3 (IQR 8.3-20.8) years, 1,176 individuals developed dementia, of whom 935 (79.5%) were diagnosed with Alzheimer's disease, 95 (8.1%) vascular dementia, 43 (3.7%) Parkinson's dementia, 30 (2.6%) another type of dementia, and in 73 (6.2%) no definite subdiagnosis could be made. Of all incident dementia cases, 129 were preceded by a stroke, a median 3.7 years (IQR 1.2-7.2) before diagnosis of dementia.

Orthostatic hypotension at baseline was associated with an increased risk of dementia during follow-up (adjusted hazard ratio [95% confidence interval]: 1.15 [1.00-1.34], *P*=0.05; Table 2). Similarly on a continuous scale, variability in systolic blood pressure related to postural change was associated with an increased risk of dementia (HR per SD increase: 1.08 [1.01-1.16], *P*=0.02). This association was similar when excluding those who fulfilled the formal criteria for orthostatic hypotension (HR 1.08 [1.00-1.17], *P*=0.06), and unaltered by excluding those with a marked increase in blood pressure following postural change (Table 3). Results were similar for Alzheimer's disease only. For vascular dementia, we observed higher risk estimates with orthostatic hypotension in an age- and sex-adjusted model (HR 1.53 [0.97-2.43], *P*=0.07), but these were largely explained by cardiovascular risk factors, so that fully adjusted estimates were similar to those for Alzheimer's disease (Table 2).

We did not observe a clear exposure-response relation for severity of orthostatic hypotension, due to lower effect estimates for participants with the most severe blood pressure drops (Figure 2). In contrast, risk of dementia did strongly increase per quartile of blood pressure variability (Figure 2). Risk estimates were similar when modelling dementia with death as a joint outcome (adjusted HR [95% CI] for orthostatic hypotension: 1.17 [1.08-1.27], *P*<0.001; and for blood pressure variability: 1.08 [1.04-1.12], *P*<0.001). Estimates for both orthostatic hypotension and systolic blood pressure variability were attenuated when incorporating these simultaneously in a model (adjusted HR [95% CI] for orthostatic hypotension: 1.07 [0.90-1.27], and for blood pressure variability: 1.06 [0.99-1.14]).

	All-cause dementia $N_{dementia}$**=1176** HR (95% CI)	**Alzheimer's disease** $N_{dementia}$**=935** HR (95% CI)	**Vascular dementia** $N_{dementia}$**=95** HR (95% CI)
Model I			
Orthostatic hypotension (yes versus no)	1.14 (0.99-1.31)	1.11 (0.95-1.30)	1.53 (0.97-2.43)
Systolic blood pressure variability (per SD)	1.07 (1.00-1.14)	1.10 (1.03-1.18)	0.93 (0.76-1.13)
Model II			
Orthostatic hypotension (yes versus no)	1.15 (1.00-1.34)	1.17 (0.99-1.37)	1.20 (0.73-1.96)
Systolic blood pressure variability (per SD)	1.08 (1.01-1.16)	1.11 (1.04-1.20)	0.92 (0.76-1.13)

Table 2. Orthostatic hypotension and risk of dementia. Model I is adjusted for age and sex, and model II additionally for blood pressure, antihypertensive medication, diabetes, total and HDL cholesterol, lipid-lowering medication, smoking, alcohol consumption, anti-cholinergic medication, and *APOE* genotype. SD=per standard deviation increase in the coefficient of variation; HR=hazard ratio; CI=confidence interval.

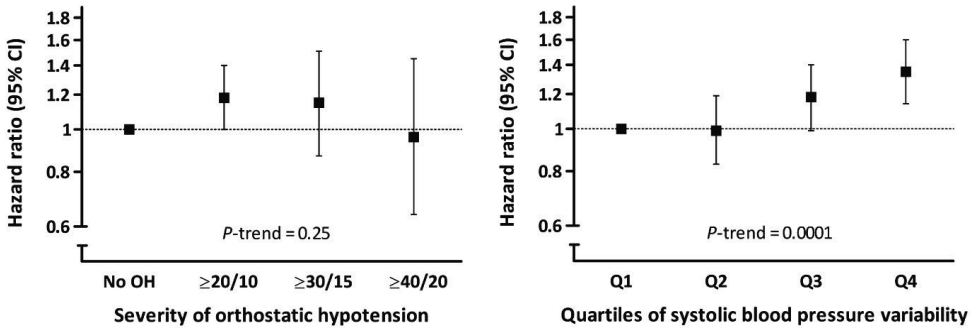


Figure 2. The severity of orthostatic blood pressure changes. The risk of dementia is depicted by severity of the orthostatic blood pressure drop in mmHg (left), and per quartile of systolic blood pressure variability (right).

SBP variability	Participants without orthostatic hypotension HR (95% CI)	Participants without strong blood pressure increase* HR (95% CI)
Per quartile		
Lowest quartile	REFERENCE	REFERENCE
2nd quartile	0.97 (0.80-1.17)	1.00 (0.82-1.21)
3rd quartile	1.18 (0.98-1.42)	1.14 (0.95-1.38)
Highest quartile	1.46 (1.18-1.81)	1.31 (1.09-1.57)
Per standard deviation	1.08 (1.00-1.17)	1.08 (1.01-1.15)

Table 3. Systolic blood pressure variability and dementia risk. Results are presented for the fully adjusted model. SBP=systolic blood pressure, with variability expressed as the coefficient of variation; HR=hazard ratio; CI=confidence interval; *Defined as ≥20mmHg systolic or ≥10mmHg diastolic increase.

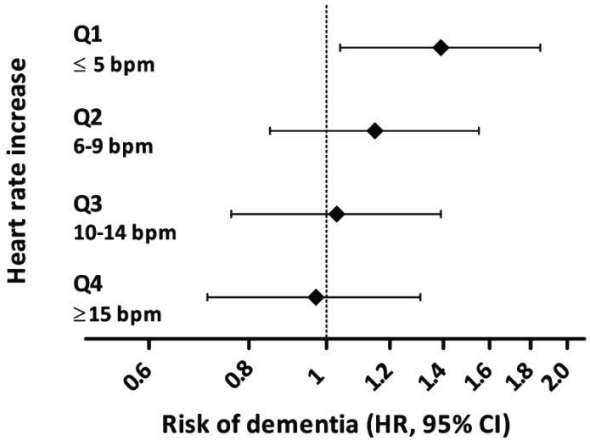


Figure 3. Heart rate response in orthostatic hypotension. The relative risk of dementia with orthostatic hypotension is presented by quartiles of orthostatic rise in heart rate. Bpm=beats per minute

Results for orthostatic hypotension were similar for badly tolerated blood pressure drops (i.e. participants reported feeling unwell) compared to subjectively well-tolerated blood pressure drops (HR 1.20 [0.86-1.66] versus 1.15 [0.98-1.34], respectively). The risk of dementia related to orthostatic hypotension was most profound in participants who lacked compensatory increase in heart rate (within the lowest quartile of heart rate response: HR 1.39 [1.04-1.85]; *P*-value for interaction = 0.05, Figure 3). This was similar after excluding all participants taking beta-blockers.

Sensitivity analyses showed similar results after censoring for incident stroke, excluding participants with prevalent Parkinson's disease, excluding those with possible postural tachycardia syndrome, or omitting the first 5 years of follow-up (Table 4). A history of hypertension or use of any antihypertensive medication did not modify the risk of dementia associated with orthostatic hypotension (Table 4). Amongst 177 participants with heart failure at baseline, risk estimates for orthostatic hypotension were higher than in those without heart failure, albeit not statistically significant (HR 1.52 [0.63-3.66]; *P*-value for interaction = 0.07).

	$N_{dementia}/N_{total}$	HR (95% CI)
Censoring for incident stroke	1001/5929	1.18 (1.01-1.38)
Excluding history of Parkinson's disease	1076/5704	1.16 (1.00-1.35)
Excluding history of heart disease*	946/5018	1.28 (1.09-1.50)
Excluding possible postural tachycardia syndrome**	1104/5775	1.18 (1.02-1.37)
Excluding the first 5 years of follow-up	882/5081	1.22 (1.03-1.44)
Sex		
Male	344/2415	1.04 (0.77-1.41)
Female	784/3514	1.19 (1.00-1.40)
Age (stratified by median)		
<68.5 years	388/3186	1.05 (0.78-1.41)
≥68.5 years	740/2742	1.16 (0.98-1.38)
Heart failure		
No	1089/5685	1.13 (0.97-1.31)
Yes	30/177	1.52 (0.63-3.66)
Hypertension		
No	469/2674	1.22 (0.96-1.55)
Yes	657/3244	1.12 (0.93-1.36)
Antihypertensive medication		
None	768/4104	1.13 (0.94-1.36)
Any anti-hypertensive drug(s)	360/1825	1.15 (0.90-1.47)
Diabetes		
No	958/5018	1.12 (0.94-1.34)
Yes	170/911	1.35 (0.74-2.47)

Table 4. Subgroup analyses for the risk with orthostatic hypotension. HR=hazard ratio; CI=confidence interval; * includes myocardial infarction, heart failure, and atrial fibrillation; ** defined as ≥30 beats per minute increase in heart rate, or any heart rate ≥120 beats per minute. Hazard ratios are presented for the fully adjusted model.

DISCUSSION

In this large population-based study, orthostatic hypotension was present in nearly 1 in 5 participants, and associated with a 15% increase in long-term risk of dementia. The risk of developing dementia was highest in those with orthostatic hypotension lacking compensatory increase in heart rate. Similarly, higher variability in blood pressure related to postural change, was associated with a higher risk of dementia, even in those persons without a formal diagnosis of orthostatic hypotension.

Prevalence of orthostatic hypotension in our study was high, and increased steeply with age, in line with previous studies among community-dwelling individuals of similar age.[7,8] A few studies have investigated orthostatic hypotension in relation to cognitive test performance. In the ARIC study, orthostatic hypotension was associated with decline on two cognitive tests, but this was largely explained by cardiovascular risk factors.[22] Two smaller studies found no overall association between orthostatic hypotension and decline on the mini-mental state examination after two years.[7,23] Conversely, orthostatic hypotension was found to increase the risk of conversion from mild cognitive impairment to dementia after 3 years,[24] as well as the risk of dementia in patients with Parkinson's disease.[25] Only one other study has assessed the relation between orthostatic hypotension and the risk of dementia in initially healthy individuals. In a sample of 1480 individuals of the Swedish general population, OH was associated with the risk of having dementia at re-examination after 6 years.[14] However, study design hampered the use of survival models, or adjustment for (cardiovascular) risk factors aside hypertension, and attrition was substantial with 37.5% of participants lost to follow-up.[14] We found orthostatic hypotension to be associated with long-term risk of dementia on continuous follow-up, independent of various other risk factors.

The most apparent explanation for our findings is that orthostatic hypotension causes brain damage due to recurrent transient cerebral hypoperfusion. Autonomic nervous system function is responsible for maintaining continuous cerebral perfusion together with local vasoreactivity, which is also associated with dementia risk in the general population.[6] Brief episodes of hypoperfusion, elicited by sudden blood pressure drops, may lead to hypoxia with detrimental effects on brain tissue via for instance neuroinflammation and oxidative stress.[26] These mechanisms have been suggested of particular relevance in the pathogenesis of small-vessel disease,[27] and orthostatic blood pressure drops in patients with dementia have been associated with deep white matter and basal ganglia hyperintensities,[28] albeit not overall white matter lesion volume.[29] The reduction in cerebral blood flow with autonomic failure has also been reported to predominantly affect the hippocampus,[30] possibly linking

hypoperfusion to early Alzheimer's pathology. Another potential explanation for our findings is that orthostatic hypotension serves as a marker of wider autonomic dysfunction. The extensive follow-up duration of our study limits the risk of reverse causation, but manifestations of autonomic dysfunction such as blood pressure variability,[31,32] response to Valsalva manoeuvre,[13,33] cardiovascular reflex and heart rate variability,[34-36] and 30/15 ratio,[35] may be linked to dementia physiologically independent of orthostatic pressure changes. The similar associations with postural blood pressure variability in individuals without orthostatic hypotension in our study may in that context represent evidence of wider autonomic failure, as much as it could mean that only subtle blood pressure drops can be clinically meaningful in the long run. Similarly, the stronger risk estimates with limited to no heart rate increase, could point to physiological cerebral blood flow impairment, or again autonomic failure in general. Future studies are encouraged to incorporate various expressions of autonomic dysfunction measured in the same individuals simultaneously, both in observational setting for determining associated risks, as well as in intervention studies to disentangle the mechanisms, for example by assessing the cerebral haemodynamic consequences of orthostatic changes in blood pressure and heart rate using near-infrared spectroscopy or transcranial Doppler.

The risk of dementia associated with orthostatic hypotension in our study was independent of how well blood pressure drops were tolerated by participants, and the vast majority of patients with orthostatic hypotension did not have symptoms during testing. This suggests that formal assessment of orthostatic hypotension is necessary to provide sufficient test sensitivity to be used in clinical practice. Hypotension might be harmful even without accompanying clinical symptoms such as light-headedness. This lack of symptoms with orthostasis was previously observed in patients with dementia,[37] and may warrant caution in view of studies linking low blood pressure in late-life to cognitive decline and dementia.[38] Although for blood pressure variability we observed an exposure-response association, we did not find this for severity of orthostatic hypotension itself. As orthostatic hypotension is also associated with mortality,[9] this may be attributable to competing risk, causing the most severely affected participants to die at a younger age, prior to developing dementia.

Orthostatic hypotension most commonly arises due to autonomic dysfunction in the absence of neurological disease, but may be provoked by synucleinopathies (e.g. Parkinson's disease), small fibre peripheral neuropathy, volume depletion (e.g. due to diuretics), and diminished cardiac pump function. In addition, several drugs can cause or aggravate orthostatic hypotension, including antihypertensive agents and antidepressants. Participants in our study with heart failure at baseline seemed particularly affected by orthostatic hypotension, possibly due to lack of compensatory increase in stroke volume. Orthostatic

hypotension has been associated with development of structural cardiac changes, including left ventricular hypertrophy,[39] which may function as a mediator towards dementia.[40] However, the subgroup of participants with heart failure in our study was too small to draw any firm conclusions. We found similar associations between orthostatic hypotension and dementia after excluding those with Parkinson's disease, and in users versus non-users of antihypertensive medication.

Although we believe our findings are valid, there are certain limitations to our study to take into account. First, despite a 25-year follow-up period with similar risk estimates over time, subclinical brain changes leading to dementia occur years if not decades prior to onset of clinical symptoms, and we can therefore not completely rule out reverse causality influencing our findings. Second, despite adjustment for many potentially confounding factors, residual confounding may persist, in particular in case of prolonged exposure to risk factors since mid-life, which was not assessed. Third, we continued blood pressure measurements for up to three minutes after postural change, and while in line with international guidelines, this may have resulted in missed orthostatic blood pressure drops beyond this time window.[41] However, any misclassification (i.e. missed diagnosis of orthostatic hypotension) would likely have led to underestimation of the true effect. Fourth, we were unable to adjust for the fact that orthostatic hypotension predisposes for falls, which may contribute to cognitive decline due to traumatic brain injury. Finally, the majority of our study population was of Caucasian descent, and findings may not be applicable to other ethnicities.

In conclusion, orthostatic hypotension is associated with an increased risk of dementia in this population-based cohort. This supports an important role of maintaining continuous cerebral perfusion in the prevention of dementia.

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Chapter 3.3

Cerebrovascular reactivity

ABSTRACT

Cerebrovascular reactivity is a key factor in the regulation and maintenance of continuous cerebral perfusion. Impaired autoregulation may lead to transient episodes of hypoxia, with potential detrimental consequences to neuronal health. Several clinical studies have reported lower vasoreactivity in patients with dementia and mild cognitive impairment than in healthy controls, but whether impaired vasoreactivity predisposes to the development of dementia is undetermined. We measured cerebrovascular reactivity in 1629 non-demented, stroke-free participants (mean age 71 years, 46% female) of the population-based Rotterdam Study, who underwent transcranial Doppler with induced hypercapnia between 1997 and 1999. We used a Cox model to determine the risk of dementia, adjusted for age, sex, and cardiovascular risk factors including carotid intima-media thickness. We also determined change in cognitive test performance in relation to vasoreactivity, using linear mixed models. During a mean follow-up of 11.5 years, 209 participants were diagnosed with dementia, of whom 171 had Alzheimer's disease. Higher vasoreactivity at baseline was associated with lower risk of dementia (hazard ratio [95% confidence interval] per standard deviation increase: 0.87 [0.75-1.00]), including Alzheimer's disease (HR 0.84 [0.71-0.99]). Risk estimates were highest in individuals without hypertension (HR 0.69 [0.53-0.91] versus 0.95 [0.79-1.14] in those with hypertension; *P*-value for interaction = 0.03). Participants with higher vasoreactivity performed better on cognitive tests at baseline (*g*-factor: β=0.063, *P*=0.007), but vasoreactivity was not associated with change in test performance during three consecutive assessments over 11 years of follow-up (*g*-factor: β=-0.021, *P*=0.34), irrespective of hypertensive status. In conclusion, impaired cerebrovascular reactivity is associated with an increased risk of dementia in the general population, suggesting that transient episodes of cerebral hypoxia due to failing autoregulation may contribute to the development of dementia.

INTRODUCTION

Cardiovascular health is an important determinant in the prevention of dementia, including Alzheimer's disease,[1,2] but studies have thus far not been able to identify the key underlying pathways. The cerebral microvasculature is widely implicated in the disease process,[3] but studies have generally relied on static markers of cerebrovascular pathology, such as small vessel disease on MRI, and insight in functional cerebral haemodynamics is therefore sparse. This is of particular relevance in light of recent studies linking (transient) changes in cerebral perfusion to dementia risk,[4-7] suggesting cerebral autoregulatory mechanisms could be vital for neuronal function and survival.

Cerebrovascular reactivity reflects the ability of the cerebral arterioles and capillaries to dilate in response to increased neuronal metabolic demand,[8] and is largely responsible for maintenance of continuous cerebral perfusion. Quantified in vivo using transcranial Doppler or MRI, impaired vasoreactivity has been associated with (cardiovascular) mortality in the general population,[9] and risk of stroke in the presence of flow-limiting carotid artery stenosis.[10] Several small cross-sectional studies have furthermore found reduced cerebrovascular reactivity in patients with dementia or mild cognitive impairment compared to healthy controls,[11-13] but its effect on cognitive decline and the risk of developing dementia is yet undetermined. We hypothesised that impaired cerebrovascular reactivity increases the risk of dementia, and aimed to determine the association of vasoreactivity with cognitive decline and dementia risk in a population-based study.

METHODS

Study population

This study is embedded within the Rotterdam study, an ongoing population-based cohort study in the Netherlands, with an initial study population of 7,983 participants aged ≥55 years from the Ommoord area, a suburb of Rotterdam. The Rotterdam study methods have been described previously.[14] Briefly, participants were interviewed at home and subsequently examined at the research centre for baseline assessment from 1990 to 1993. Until 2013, four follow-up examinations have been carried out. Transcranial Doppler (TCD) investigation with induction of hypercapnia was added to the core protocol for the second follow-up examination, from July 1997 to December 1999. Of 5,990 survivors from the original cohort, 4,797 participated in this follow-up, of whom 4,215 visited the study centre for examination. Due to lack of technical support and personnel, cerebrovascular reactivity could be measured in a random subset of 2,731 of these participants.

Transcranial Doppler (TCD) assessment

TCD monitoring was performed (Multi-Dop X-4; DWL, Sipplingen, Germany) and the cerebral blood flow velocity (cm/sec) was measured in the middle cerebral artery on both sides. End-diastolic, peak systolic, and mean flow velocities were recorded automatically. End-tidal CO_2 pressure (kPa) was recorded continuously with a CO_2 analyzer (Multinex; Datascope, Hoevelaken, the Netherlands). Cerebral CO_2 vasoreactivity (CVR) was determined by continuous measurement of flow velocity in the middle cerebral artery, while participants breathed room air followed by 5% carbon dioxide inspiration through an anaesthetic mask for 2 minutes. CVR was defined as the percentage increase in flow velocity during inspiration of 5% CO_2, divided by the absolute increase in end-tidal CO_2 in the same period. We used the mean of right and left hemodynamic parameters for the analyses. In case of one-sided window absence, the contralateral parameters were used for analyses. Blood pressure was measured before and at the end of 5% CO_2 inspiration, to adjust for mean arterial pressure related change in end-tidal CO_2.

Dementia screening and surveillance

Participants were screened for dementia at baseline and subsequent centre visits using the Mini-Mental State Examination (MMSE) and the Geriatric Mental State Schedule (GMS) organic level.[15] Those with MMSE<26 or GMS>0 underwent further investigation and informant interview including the Cambridge Examination for Mental Disorders of the Elderly. Additionally, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study centre with medical records from general practitioners and the regional institute for outpatient mental healthcare. Available clinical neuroimaging data were reviewed when required for diagnosis of dementia subtype. A consensus panel headed by a consultant neurologist established the final diagnosis according to standard criteria for dementia (DSM-III-R), and Alzheimer's disease (NINCDS-ADRDA).

Cognitive function assessment

Cognitive function was assessed in detail with a test battery comprising the Stroop test (time in seconds taken for completing the reading/colour naming interference task), the letter-digit substitution task (number of correct digits in 1 minute), and the verbal fluency test (number of animal species within 1 minute).[16] Cognitive function assessment was carried out at baseline (time of TCD) and at two subsequent follow-up examinations. To obtain an overall measure of cognitive tests, we calculated the *g*-factor, which explained 62-64% of the overall variance in cognitive test scores in our population. For each participant, z-scores were calculated for each test separately, by dividing the difference between individual test score and mean test score by the standard deviation.

Other measurements

We assessed smoking status (i.e. current, former, never) and medication use at baseline by interview. Blood pressure was measured on the right arm with a random-zero sphygmomanometer prior to and during TCD investigation; hypertension was defined as a systolic/diastolic blood pressure >140/90mmHg, or the use of antihypertensive medication. Fasting serum lipid levels were measured at baseline. Diabetes was defined as the use of blood glucose-lowering medication at baseline or a fasting serum glucose level ≥7.0 mmol/L. Carotid intima media thickness was measured by Doppler ultrasound. *APOE* genotype was determined using polymerase chain reaction on coded DNA samples, and carrier status defined as heterozygote (1 ε4 allele) or homozygote (2 ε4 alleles).

Analysis

Analyses included all non-demented participants without a history of stroke, who underwent TCD. Because of a right-skewed distribution of CVR, we first performed a natural logarithmic transformation to obtain a roughly normal distribution of the data. Missing covariate data (maximum 8.3%) were imputed using 5-fold multiple imputation, based on determinant, outcome and included covariates (with *APOE* genotype as predictor only). Distribution of covariates was similar in the imputed versus non-imputed dataset. We used analysis of covariance (ANCOVA) to test for age- and sex-adjusted differences in baseline characteristics between participants who underwent TCD and those who did not.

We assessed the association between CVR and various cardiovascular risk factors, using linear regression. We then determined risk of dementia and Alzheimer's disease by time following TCD assessment, using Cox proportional hazard models. The proportional hazard assumption was met. We used follow-up time in years as the time-scale in these models, and verified that the choice of time scale (time on study versus age of onset) did not affect the results. Follow-up was near complete till 1st January 2014 (95.7% of potential person years), and participants were censored within this follow-up period at date of dementia diagnosis, date of death, date of loss to follow-up, or 1st January 2014, whichever came first. We repeated analyses 1) for men and women separately; 2) for persons above and below the median age of 70 years; 3) censoring for incident stroke; 4) excluding participants with exhausted vasomotor reactivity (i.e. values below -2SD from the mean) as may be seen in case of severe carotid artery stenosis or occlusion,[17] and 5) excluding the (arbitrarily chosen) first 4 years of follow-up to assess potential reverse causality. We assessed effect modification by baseline blood pressure, and by change in mean arterial pressure during investigation by adding multiplicative interaction terms to the model, and stratification.

Next, we determined the association between baseline CVR and baseline test scores on the cognitive assessment battery, as well as decline in test scores during follow-up, using linear mixed models. We fitted the model in maximum likelihood to the *g*-factor of scores on the cognitive assessment battery. Based on the Bayesian information criterion (BIC), we chose a Toeplitz with homogenous variance structure as covariance structure for the fixed effects, and made no assumptions (unstructured) for the random effects. Adding a quadratic term did not improve the model. Next, we simplified the saturated model by excluding redundant interactions between covariates, again based on the BIC, resulting in a model with the interactions follow-up*age and follow-up*CVR. Finally, we added other covariates in agreement with the fully adjusted model for dementia, and refitted the model in restricted maximum likelihood.

All analyses were adjusted for age, sex, and change in mean arterial pressure following hypercapnia, and additionally in a second model for blood pressure, serum total cholesterol, HDL cholesterol and triglycerides, use of antihypertensive or lipid-lowering medication, diabetes, carotid intima-media thickness, and *APOE* genotype. Analyses were done using SPSS Statistics version 21 (IBM Corp, Armonk, NY, USA). Alpha (type 1 error) was set at 0.05.

Characteristics	**Participants (*N*=1629)**	**Non-participants (*N*=940)**	**P-value***
Age (mean±SD)	70.6 ±6.2	72.9 ±6.7	<0.0001
Female sex	754 (46.3%)	713 (75.9%)	<0.0001
Smoking			
Former	908 (56.1%)	381 (41.0%)	0.005
Current	257 (15.9%)	146 (15.7%)	0.11
Systolic blood pressure (mm Hg, mean±SD)	143 (±21)	144 (±21)	0.47
Diastolic blood pressure (mm Hg, mean±SD)	76 (±11)	75 (±11)	0.91
Blood pressure lowering medication	579 (36.4%)	384 (42.4%)	0.14
Diabetes	193 (12.2%)	126 (14.0%)	0.13
Total cholesterol (mean±SD)	5.8 (±1.0)	5.9 (±1.0)	0.20
HDL cholesterol (mean±SD)	1.4 (±0.4)	1.4 (±0.4)	0.11
Triglycerides (mean±SD)	1.5 (±0.8)	1.6 (±0.7)	0.19
Lipid-lowering medication	223 (13.8%)	135 (14.7%)	0.26
Body mass index (mean±SD)	26.6 (±3.8)	27.3 (±4.3)	0.007
Carotid intima-media thickness (mm, mean±SD)	1.06 (±0.18)	1.08 (±0.19)	0.06
APOE genotype			
ε4 heterozygosity	438 (28.0%)	228 (25.6%)	0.28
ε4 homozygosity	35 (2.2%)	17 (1.9%)	0.73
Transcranial Doppler investigation			
CO_2 vasoreactivity (%/kPa, median, IQR)	39.6 (28.6-53.9)	n/a	-
ΔMAP during CO_2 challenge (mmHg, mean±SD)	8.8 (±7.3)	n/a	-

Table 1. Baseline characteristics of participants and non-participants. SD=standard deviation; IQR=interquartile range; MAP=mean arterial pressure; n/a=not applicable. *adjusted for age and sex when applicable.

RESULTS

Among 2,569 eligible participants undergoing TCD with induced hypercapnia, no temporal bone window was present on either side in 632 (24.6%) individuals. Measurements could not be completed in 214 (8.3%) cases, due to participants feeling anxious or unwell (n=54), lack of time (n=3), or other undocumented causes (n=157). In addition, in 94 participants we failed to obtain a reliable measurement of cerebrovascular reactivity (CVR) despite adequate CO_2 induction, thus leaving a total of 1,629 cases for analysis. Baseline characteristics of participants in comparison with non-participants are presented in Table 1.

CVR was lower in women than in men, and also impaired in current smokers, individuals with dyslipidaemia, and to a lesser extent those with diabetes. Conversely, higher blood pressure at time of examination and higher BMI were significantly associated with higher CVR, as were more pronounced increases in mean arterial pressure (MAP) in response to the CO_2 challenge (Table 2).

	β (95% CI)	***P*-value**
Age (per 10 years)	-0.223 (-0.302;-0.144)	<0.0001
Female sex	-0.213 (-0.327;-0.098)	0.0003
Smoking		
Never	REFERENCE	
Former	-0.087 (-0.206;0.032)	0.15
Current	-0.314 (-0.471;-0.157)	<0.0001
Mean arterial pressure (per 10mmHg)	0.096 (0.060;0.132)	<0.0001
Δ Mean arterial pressure during CO_2 challenge	0.156 (0.087;0.225)	<0.0001
Antihypertensive medication	-0.066 (-0.175;0.042)	0.23
Diabetes	-0.139 (-0.291;0.013)	0.07
Cholesterol (per mmol/L)	-0.051 (-0.107;0.004)	0.07
High-density lipoprotein (per mmol/L)	0.225 (0.063;0.387)	0.007
Triglycerides (per mmol/L)	0.069 (-0.011;0.148)	0.09
Lipid-lowering medication	-0.098 (-0.247;0.051)	0.20
Body-mass index (per 1 point increase)	0.025 (0.011;0.038)	0.0004

Table 2. Cardiovascular risk factors and cerebrovascular reactivity. All presented variables were entered in the multivariable model.

During a mean follow-up of 11.5 (±4.3) years, 209 individuals developed dementia, of whom 171 (81.2%) had Alzheimer's disease. Lower CVR at baseline was associated with an increased risk of dementia during follow-up, similar for all-cause dementia and Alzheimer's disease (Figure 1). Of all incident dementia cases, 30 were preceded by a stroke (a median 4.5 years before dementia diagnosis), but censoring at time of stroke did not affect risk estimates of CVR for dementia (Figure 2). Risk estimates were also robust against excluding the first years of follow-up (Figure 2). Effects were somewhat larger in women than in men, and in younger individuals, albeit neither difference was statistically significant (Figure 2).

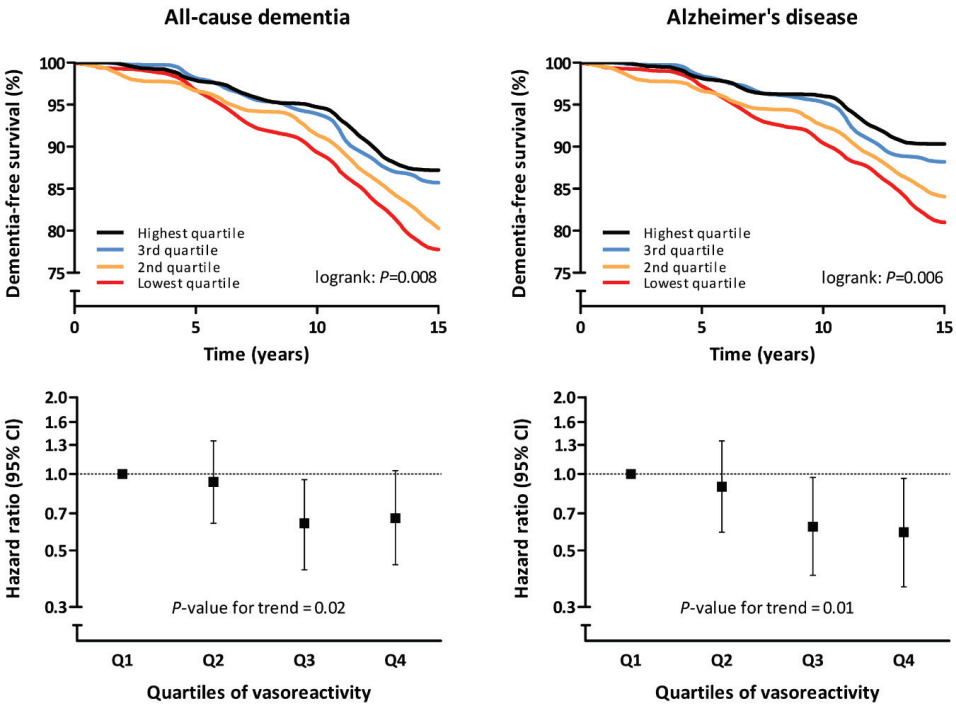

Figure 1. Risk of dementia and Alzheimer's disease. Baseline cerebrovascular reactivity in relation to risk of developing dementia (left) and Alzheimer's disease (right), visualised as dementia-free survival in a smoothed Kaplan-Meier curve (top) and per quartile of vasoreactivity in a fully adjusted Cox model (bottom).

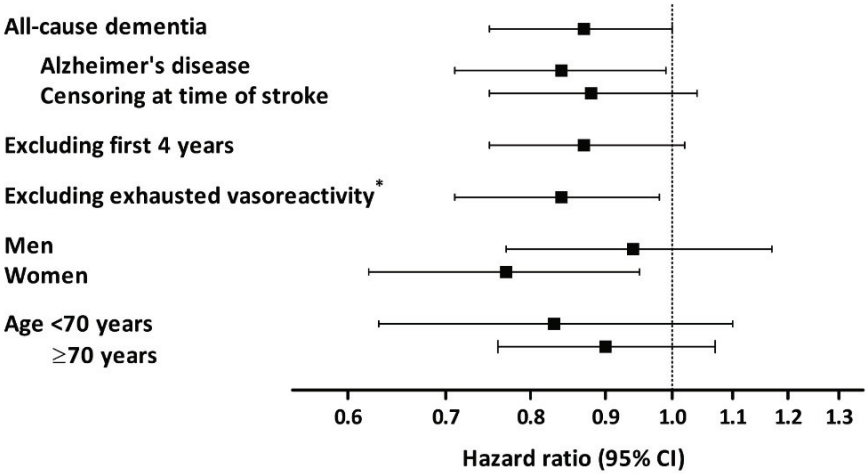

Figure 2. Subgroup and sensitivity analyses for vasoreactivity and dementia risk. The figure displays for several sensitivity analyses the relative risks for dementia per standard deviation increase in cerebrovascular reactivity. Results from the fully adjusted Cox model are shown. Exhausted vasoreactivity is defined as any value below -2 standard deviations from the mean. CI=confidence interval.

Of all participants, 1,154 (70.1%) had hypertension at baseline, of whom 579 were taking blood pressure lowering medication. Risk estimates of CVR for dementia were higher in individuals without hypertension (HR [95%CI] per standard deviation increase: 0.69 [0.53-0.91] versus 0.95 [0.79-1.14] in those with hypertension; *P*-value for interaction = 0.03). This was driven by higher estimates in individuals who had low-normal blood pressure levels without use of blood pressure lowering medication (Table 3). Risk estimates for CVR also tended to differ with change in MAP following CO_2 challenge (*P*-value for interaction = 0.08), such that risk of dementia was highest if impaired CVR was accompanied by a marked increase in MAP (e.g.HR per SD increase in CVR 0.58 [0.40-0.83] within the highest quartile of ≥13mmHg). This was seen regardless of hypertensive status, and similar when assessing relative (as a percentage of baseline MAP) rather than absolute change in MAP.

Of all participants, 1,608 (98.7%) underwent cognitive testing at baseline, and repeated assessment was done in 1,094/1,251 (87.5%) and 699/910 (76.8%) of surviving, non-demented individuals after a mean follow-up of 4.5 (SD 0.5) years and 11.0 (SD 0.3) years, respectively. Participants with higher CVR performed better on cognitive tests at baseline (*g*-factor: β=0.063, *P*=0.007), in particular due to improved performance on the letter-digit substitution task (Figure 3). CVR was not associated with change in test performance during three consecutive assessments over 11 years of follow-up (*g*-factor: β=-0.021, *P*=0.34; Figure 3), irrespective of age, sex, and hypertensive status (all *P*-values for interaction >0.05).

	No blood-pressure lowering medication		Using blood-pressure lowering medication	
	$N_{dementia}/N_{total}$	HR (95% CI)	$N_{dementia}/N_{total}$	HR (95% CI)
All participants	129/1041	0.85 (0.71-1.02)	80/588	0.93 (0.75-1.15)
Blood pressure				
SBP <130	28/259	0.66 (0.45-0.95)	7/90	1.03 (0.45-2.34)
SBP 130-149	54/389	0.88 (0.67-1.15)	29/198	1.07 (0.70-1.64)
SBP ≥150	47/393	1.01 (0.71-1.43)	44/300	0.87 (0.64-1.17)
DBP <75	48/343	0.83 (0.61-1.11)	21/177	0.92 (0.60-1.41)
DBP 75-84	45/371	0.84 (0.61-1.16)	31/228	0.89 (0.61-1.28)
DBP ≥85	36/326	0.90 (0.62-1.31)	28/183	0.94 (0.63-1.41)
MAP <95	49/351	0.77 (0.58-1.04)	13/133	1.21 (0.66-2.22)
MAP 95-104	30/286	0.95 (0.66-1.36)	27/184	0.84 (0.58-1.23)
MAP ≥105	50/394	0.86 (0.61-1.22)	40/271	0.92 (0.66-1.28)
PP <60	43/395	0.74 (0.56-0.99)	16/159	0.90 (0.50-1.63)
PP 60-74	51/368	0.86 (0.63-1.16)	26/194	0.85 (0.56-1.29)
PP ≥75	35/276	1.00 (0.67-1.50)	38/235	0.94 (0.68-1.28)

Table 3. Cerebrovascular reactivity and dementia in relation by hypertensive status. SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure; PP=pulse pressure; HR=hazard ratio; CI=confidence interval. Hazard ratios are presented per standard deviation increase in vasoreactivity for a model including age, sex, change in mean arterial pressure during CO_2 challenge, and baseline blood pressure.

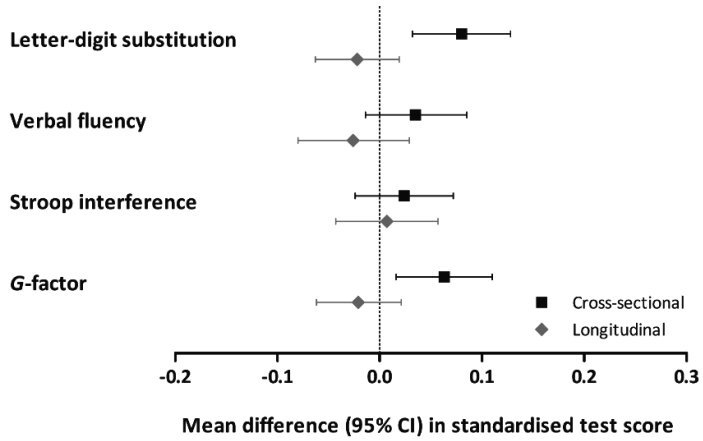

Figure 3. Cerebrovascular reactivity and cognitive test performance. The figure shows the association of cerebrovascular reactivity with cognitive test scores at baseline (cross-sectional, black boxes), and with change in cognitive scores across three cognitive examination rounds (longitudinal, grey diamonds). Results represent the mean difference in standardised test scores per standard deviation increase in vasoreactivity at baseline, and for the longitudinal analyses expressed per 10 years of follow-up.

DISCUSSION

In this population-based study, lower cerebrovascular reactivity was associated with an increased long-term risk of developing dementia and Alzheimer's disease. Participants with low vasoreactivity did wose on cognitive testing at baseline, but despite prolonged associations of vasoreactivity with dementia risk, this did not translate into less decline on repeated cognitive testing.

In line with prior cross-sectional studies,[11-13] participants with low vasoreactivity performed worse on cognitive assessment at baseline, and importantly, among cognitively healthy individuals these findings translate into an increased risk of developing dementia. The sustained risk increases beyond the first years of follow-up thereby suggests that vasoreactivity not only changes secondary to ongoing neurodegeneration, but could also play a role in its pathophysiology. Nevertheless, we did not observe the same pattern for changes in performance on a cognitive assessment battery in non-demented individuals. This could mean that impaired vasoreactivity is not necessarily harmful with an otherwise healthy brain and functioning autonomous nervous system, although methodological considerations like insensitive outcome measures or substantial attrition for repeated cognitive testing at the end of follow-up should not be discounted. In the absence of other published longitudinal studies our findings therefore warrant replication, notwithstanding their interest in light of several potential underlying mechanisms.

Cerebrovascular reactivity depends on endothelial cell, pericyte and vascular smooth muscle cell function. Endothelial vasodilators, among which noticeably nitric oxide, are important mediators of the autoregulatory response,[18-19] and they have previously been linked to dementia pathology by correlations of endothelial nitric oxide synthase levels with tau and amyloid burden.[20] Failure of the autoregulatory response leads to (episodic) hypoperfusion or uncontrolled hyperaemia, and the resultant reduction in tissue oxygenation can directly trigger expression of various inflammatory cytokines via activation of hypoxia-inducible transcription factors (HIF).[21,22] Inflammatory cytokines subsequently activate microglia,[23] inducing release of pro-inflammatory neurotoxic factors (e.g. IL-1β and TNFα) and oxidative stress. Similar rises in inflammatory factors, including TNFα, TGFβ, various interleukins and matrix-metalloproteinases (MMP), are seen in patients with Alzheimer's disease.[24] Furthermore, HIF renders endothelial cells responsive to proangiogenic factors, including vascular endothelial growth factor (VEGF), angiopoietins and platelet derived growth factor (PDGF). These factors are vital for maintaining blood-brain barrier integrity through regulating endothelial cell and pericyte function in angiogenesis,[25,26] and pericyte deficiency itself has been associated with age-related vascular damage that precedes neurodegeneration.[27] Hypoxia is furthermore found to lead to aberrant angiogenesis and microvascular degeneration in patients with Alzheimer's disease by suppressing expression of the mesenchyme homeobox 2 gene (MEOX2) in brain endothelial cells.[28] MEOX2 deficient mice in the same study showed vascular degeneration and poor amyloid-β clearance,[28] implicating MEOX2 as a mediator between hypoxia and hallmarks of Alzheimer's disease pathology.

The strongest association between vasoreactivity and dementia in our study was observed in individuals with low blood pressure and no prior treatment for hypertension, in line with the presumption that low arterial pressure renders the brain particularly vulnerable to sudden pressure drops. We would however have expected this finding to extend to the hypertensive population, as chronic hypertension shifts the regulatory range of mean arterial pressure towards higher levels,[29] protecting the brain against higher pressures, but rendering it vulnerable to hypoperfusion in case of blood pressure drops.[30] Perhaps any relation to arterial pressure is obscured in this group by a wider 'normal' regulatory range with varying degrees of longstanding hypertension, or coinciding disturbances in other autoregulatory mechanisms, notably the baroreceptor reflex.[31,32] The importance of other mechanisms may also be reflected by increases in mean arterial pressure parallel to the vasodilatory response upon carbon dioxide challenge, which could indicate physiologically insufficient dilatation of the arterioles. Finally, various antihypertensive drug classes have differential effects on (variability in) arterial pressure, and the cerebral vasculature. Minimising variability in blood pressure, for instance by calcium-channel blockers or non-loop diuretics,[33] might lessen the

challenge on autoregulatory mechanisms to maintain cerebral blood flow. However, few drugs have directly been tested for improvement of cerebrovascular reactivity, and regarding blood pressure medication in one small trial, no differences were seen in vasoreactivity between treatment with lisinopril, candesartan, and hydrochlorothiazide.[34] In view of the large risk of confounding by indication in observational studies, such (cross-over) trials could be most helpful to increase insight into physiological mechanisms, and determine optimal therapy in patients at excess risk of stroke and dementia.

Although we believe our results are valid, there are certain limitations to our study to take into account. First, the sample of the Rotterdam Study cohort that underwent TCD were older, more often female, and less often prior smokers, potentially giving rise to selection bias. As female sex and increasing age are associated with lower cerebrovascular reactivity and higher incidence of dementia,[35] this might have caused underestimation of the true population effect. Second, risk estimates were robust to adjustment for a wide range of potential cardiovascular confounders, but in the absence of brain imaging we cannot rule out residual confounding by effects of cerebral small-vessel on dementia risk other than via cerebral autoregulation.[36] Third, although follow-up for dementia was near-complete (96%), attrition for extensive cognitive assessment was substantial, potentially biasing results to the null. Fourth, we may have failed to detect certain Alzheimer specific changes, because the memory domain was not included in our cognitive assessment, and medial cerebral artery rather than posterior cerebral artery flow velocity is less informative about changes in the hippocampus and amygdala. More generally, region-specific assessment of vasoreactivity, for instance using arterial spin labelling, is likely more sensitive in detecting neurodegenerative changes.[12] Finally, cerebral blood flow regulation is a complex interplay of various mechanisms, and future research may improve upon our study by also incorporating other haemodynamic parameters such as heart rate (variability) and baroreflex sensitivity.

In conclusion, cerebrovascular reactivity is associated with an increased risk of dementia in the general population. This suggests that transient episodes of cerebral hypoxia due to impaired autoregulation may contribute to the development of dementia.

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Chapter 3.4

Haemoglobin

ABSTRACT

Disturbances in haemoglobin homeostasis are common, with anaemia alone affecting 1.6 billion people worldwide, but its effects on brain health remain largely undetermined. We aimed to determine the long-term association of haemoglobin levels and anaemia with risk of dementia, and explored underlying substrates on brain MRI in the general population. We measured serum haemoglobin levels in 12,305 non-demented participants of the population-based Rotterdam Study (mean age 65 years, 58% female). We determined risk of dementia and Alzheimer's disease (until 2016) in relation to haemoglobin and anaemia. Among 5,267 non-demented participants with brain MRI, we assessed haemoglobin in relation to vascular brain disease, structural connectivity, and global cerebral perfusion. During a mean follow-up of 12.1 years, 1,520 individuals developed dementia, of whom 1,194 had Alzheimer's disease. We observed a U-shaped association between haemoglobin levels and dementia (P=0.005), such that both low and high haemoglobin levels were associated with increased risk of dementia (adjusted hazard ratio [95% confidence interval]– lowest versus middle quintile 1.29 [1.09-1.52]; highest versus middle quintile 1.20 [1.00-1.44]). Overall prevalence of anaemia was 6.1%, and anaemia was associated with a 34% increased risk of dementia (95%CI 11-62%) and 41% (15-74%) for Alzheimer's disease. Among 5,267 non-demented individuals with brain MRI, similar U-shaped associations were seen of haemoglobin levels with volume of white matter hyperintensities (P=0.03) and structural connectivity (e.g. for mean diffusivity, P<0.0001), but not with the presence of cortical and lacunar infarcts. Cerebral microbleeds were more common with anaemia. Haemoglobin levels were inversely correlated to cerebral perfusion (difference in mL/100mL/min perfusion [95%CI] per mmol/L decrease: 3.2 [2.8-3.6]; P<0.0001). In conclusion, low and high levels of haemoglobin are associated with an increased risk of dementia, including Alzheimer's disease.

INTRODUCTION

Low levels of haemoglobin are a major global health problem affecting 1.6 billion people worldwide,[1] with prevalence of anaemia ranging from around 10% after age 65 in Europe and the Americas,[2,3] to up to 45% in African and South-east Asian countries.[1] Low haemoglobin levels (or the equivalent measure of haematocrit) have been associated with various adverse health outcomes, including coronary heart disease,[4,5] stroke,[5,6] and mortality.[7,8] Limited data, however, are available about the relation of haemoglobin with risk of dementia, and Alzheimer's disease as its most common subtype. As the prevalence of dementia is expected to increase threefold over the next decades, with the largest increases predicted in the countries where the prevalence of anaemia is highest,[9] it is crucial to acquire more insight in potential long-term effects of abnormal haemoglobin levels on brain health.

Of particular interest to Alzheimer's disease is the binding capacity of haemoglobin to amyloid-β42, a pathologic hallmark of Alzheimer's disease, via the iron-containing haem site.[10,11] Haemoglobin co-localises with the plaques and vascular amyloid deposits in the brains of patients with Alzheimer's disease,[11] and affinity of haemoglobin for binding amyloid might even influence risk of developing Alzheimer's disease.[10] Three prospective cohort studies have thus far investigated haemoglobin levels in relation to incident dementia in the population.[12-14] Each found an increased risk of dementia with anaemia, but with the average follow-up limited to three years in two out of three studies,[12,13] reverse causality due to (dietary or metabolic) changes in the pre-diagnostic phase of dementia could account for part of this association. In addition, only one study assessed the risk of dementia across the full range of haemoglobin, reporting an increase in risk of Alzheimer's disease also with high haemoglobin levels,[13] in line with risks of cardiovascular disease.[4-6] These findings merit study of haemoglobin levels, rather than anaemia alone, and dementia risk during long-term follow-up in the population. To advance insight in the yet elusive underlying pathways, concurrent brain imaging could complement these studies towards better understanding of a physiological contribution of haemoglobin to brain health.

We therefore determined the association between haemoglobin levels and risk of dementia in a population-based cohort, and explored potential substrates by studying imaging markers of vascular brain disease, structural connectivity, and cerebral perfusion.

METHODS

Study population

This study is embedded within the Rotterdam Study, a large ongoing population-based cohort study in the Netherlands, details of which have been described previously.[15] In brief, the initial study population in 1990 consisted of 7,983 participants aged ≥55 years from the Ommoord area, a suburb of Rotterdam. The cohort was subsequently expanded twice, first in 1999 including an additional 3,011 individuals who had reached age 55 or moved into the study area, and again in 2005 with 3,932 individuals from the same area aged 45 or over. Participants undergo extensive interview and follow-up examinations at a dedicated research centre every 4 years. For the present study, the baseline examination was the first examination of the original cohort and expansion cohorts (1990-1993, 1999-2001, and 2005-2008, respectively). Of the total number of 14,926 participants, 13,498 (90.4%) visited the research centre for physical examination. We excluded 396 participants because of prevalent dementia, 73 participants because of insufficient cognitive screening to assess dementia at baseline, and 98 participants who did not provide informed consent for follow-up data monitoring, leaving 13,029 (87.3%) participants eligible for this study. From August 2005 onwards, brain magnetic resonance imaging (MRI) was incorporated in the core study protocol, and until 2014, 5,319 of eligible participants underwent MRI.

Measurement of haemoglobin and definition of anaemia

We collected non-fasting venous blood samples at baseline in EDTA tubes, and haemoglobin concentrations were measured using a colorimetric method at a wavelength of 525nm (Counter T660, Beckman Coulter, Brea, California, USA). Fasting blood samples at time of brain MRI were analysed with a novel Coulter AcT diff2 Hematology Analyzer from the same producer. In accordance with WHO criteria, anaemia was defined as having haemoglobin levels <8.1mmol/L (13g/dL) for men, and <7.5mmol/L (12g/dL) for women.[16] We further differentiated microcytic (mean corpuscular volume <80fL) from normocytic (80-95fL), and macrocytic anaemia (>95fL).

Dementia screening and surveillance

Participants were screened for dementia at baseline and subsequent centre visits using the Mini-Mental State Examination (MMSE) and the Geriatric Mental State Schedule (GMS) organic level.[17] Those with MMSE<26 or GMS>0 underwent further investigation and informant interview including the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX). Additionally, the entire cohort was continuously monitored for dementia through computerised linkage of medical records from general practitioners and the regional institute for outpatient mental healthcare with the study database. Available neuroimaging

data were used when required for diagnosis of dementia subtype. For all suspected cases of dementia, a consensus panel led by a consultant neurologist decided on the final diagnosis in accordance with standard criteria for dementia (DSM-III-R), and Alzheimer's disease (NINCDS-ADRDA). Follow-up until 1st January 2016 was near complete (96.1% of potential person years), and participants were censored within this follow-up period at date of dementia diagnosis, date of death, date of loss to follow-up, or 1st January 2016, whichever came first.

MRI scan protocol and image processing

MRI of the brain was performed on a 1.5 T scanner (General Electric Healthcare, Milwaukee, WI, USA), using an 8-channel head coil. We acquired high-resolution axial T1-weighted sequence, proton-density-weighted (PD) sequence, fluid attenuated inversion recovery (FLAIR) sequence, and T2*-weighted gradient echo sequence. Sequence details, pre-processing steps, and the classification algorithm have been described previously.[18] Total intracranial and parenchymal volumes, and volume of white matter hyperintensities (WMH) were quantified using an automated tissue segmentation method.[18] All segmentation results were visually inspected, and manually corrected if needed. All scans were appraised by trained research physicians blinded to clinical data for the presence of cerebral microbleeds (i.e., small round-ovoid areas of signal loss on T2*-weighted images), lacunar infarcts (i.e., focal lesions ≥3 and <15 mm with signal intensity similar to that of CSF on all sequences and, when supratentorial, with a hyperintense rim on FLAIR), and cortical infarcts.

From March 2006 onwards, a diffusion-weighted echo-planar imaging sequence was incorporated in the scan protocol,[19] with the maximum b-value of 1000 s/mm2 in 25 non-collinear directions; three volumes were acquired without diffusion weighting (b-value = 0 s/mm2). All diffusion data were pre-processed using a standardised pipeline. In short, eddy current and head-motion correction were performed on the acquired data, and subsequently used to fit diffusion tensors to compute mean global fractional anisotropy (FA) and mean diffusivity (MD) in the normal-appearing white matter. In general, lower FA and higher MD values are considered indicative of lower structural connectivity.

For flow measurement, 2D phase-contrast imaging was performed as described previously.[20] On a sagittal 2D phase-contrast angiographic scout image, a transverse imaging plane perpendicular to both the pre-cavernous portion of the internal carotid arteries and the middle part of the basilar artery was chosen for a 2D gradient-echo phase-contrast sequence (repetition time=20 ms, echo time=4 ms, field of view=19 cm2, matrix=256 × 160, flip angle=8°, number of excitations=8, bandwidth=22.73 kHz, velocity encoding=120 cm/sec, slice thickness=5 mm). Acquisition time was 51 seconds and no cardiac gating was

performed. Flow was calculated from the phase-contrast images using interactive data language-based custom software (Cinetool version 4; General Electric Healthcare). Two experienced technicians drew all the manual regions of interest and performed subsequent flow measurements (inter-rater correlations >0.94 for all vessels).[20] We calculated global brain perfusion (mL/min per 100 mL) by dividing total cerebral blood flow (mL/min) by each individual's brain volume (mL) and multiplying the result by 100.

Other measurements

We assessed smoking status (i.e. current, former, never), educational attainment (i.e. primary, lower, further, and higher), and medication use at baseline by interview. Dietary intake was assessed by a self-administered checklist, followed by a structured food-frequency questionnaire interview with a trained dietician.[21] Non-fasting serum lipid levels were measured at baseline. Blood pressure were measured with a random-zero sphygmomanometer; the mean of two readings was used for analysis. Body mass index was computed from measurements of height and weight (kg/m^2). Diabetes was defined as the use of blood glucose-lowering medication at baseline or a random serum glucose level ≥11.1 mmol/L.[22] Glomerular filtration rates were estimated from age- and sex-calibrated serum creatinine levels. Heart failure was determined using a validated score, similar to the definition of heart failure of the European Society of Cardiology.[23] History of stroke, cancer, and chronic obstructive pulmonary disease were assessed by interview, and inspection of medical records. *APOE* genotype was determined using polymerase chain reaction on coded DNA samples.

Analysis

Analyses included all non-demented participants attending the study centre for baseline examination. Missing covariate data were imputed using fivefold multiple imputation, based on determinant, outcome and included covariates. In the entire cohort at baseline, this applied in particular to missing data for for alcohol intake (24.0%), dietary intake (25.0%), iron intake (25.2%), glomerular filtration rate (12.7%), whereas for all other variables missing data represented less than 4.5% of all data. Presented data in the MRI sample were virtually complete. Distribution of covariates was similar in the imputed versus the non-imputed dataset.

We assessed the overall prevalence of anaemia, and whether presence of anaemia was related to iron intake and adherence to dietary guidelines. We then determined the association between haemoglobin levels and incident dementia, using Cox proportional hazard models. In anticipation of a non-linear association, we formally assessed deviation from linearity using ANOVA, and proceeded with analyses per quintile of haemoglobin. We

verified that the proportional hazard assumption was not violated. All analyses were adjusted for age and sex (model I), and additionally for educational attainment, smoking habits, alcohol consumption, systolic and diastolic blood pressure, anti-hypertensive medication, body-mass index, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, diabetes, renal function, dietary intake, iron intake, vitamin supplements, anti-anaemic medication, hormone replacement therapy, and *APOE* genotype (model II). We repeated the analyses for Alzheimer's disease only, and after excluding individuals with potential secondary causes of erythrocytosis (haemoglobin >1.5 standard deviations above the mean, and any of the following: chronic obstructive pulmonary disease, heart failure, kidney dysfunction (eGFR<60), carcinoma, or use of anti-anaemic medication including erythropoietin and diuretics; *N*=340). To address potential reverse causality, we also repeated the analyses after stepwise exclusion of the first five years of follow-up. An estimation of the minimum strength of unmeasured confounding needed to explain away the observed risk estimate for anaemia on dementia was expressed as the E-value.[24]

We then determined the association of haemoglobin levels with white matter integrity (MD and FA), volume of WMH, cerebral microbleeds, presence of lacunar and cortical infarcts, and cerebral perfusion in the subset of participants who underwent brain MRI. Non-linear associations were assessed with cubic splines. Linear and logistic regression models were adjusted for age, sex, total intracranial volume, white matter volume (for WMH, MD, and FA), educational attainment, smoking habits, alcohol consumption, systolic and diastolic blood pressure, anti-hypertensive medication, body-mass index, total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication, diabetes, vitamin supplements, anti-anaemic medication, and *APOE* genotype. Due to restrictions in resources, renal function, dietary intake, iron intake, and C-reactive protein were assessed in less than half of participants at time of MRI. As adjustment for these variables did not modify the association between haemoglobin and dementia, we opted to not include these in the model for imaging markers. Lastly, because of previously reported interactions with cerebral perfusion,[25] we investigated interaction of haemoglobin levels with mean arterial blood pressure and volume of WMH by testing for multiplicative interaction between continuous variables.

All analyses were done using IBM SPSS Statistics version 21.0 (IBM Corp, Armonk, NY, USA), except for the restricted cubic splines models which were run in R (version 3.3.3; packages 'survival', 'visreg', and 'rms'). Alpha (type 1 error) was set at 0.05.

RESULTS

Of 13,029 eligible participants, 12,308 (94.5%) provided blood samples for measurement of haemoglobin levels. Measurement failed in 3 (0.02%) participants, leaving 12,305 (94.4%) individuals for analysis. Baseline characteristics of participants are shown in Table 1.

Characteristics	Complete cohort (N=12,305)	MRI subsample (N=5,267)
Age	64.6 ±9.5	64.4 ±10.6
Female sex	7105 (57.7%)	2912 (55.3%)
Educational attainment		
Lower	6948 (57.1%)	2466 (47.1%)
Further	3356 (27.6%)	1573 (30.1%)
Higher	1874 (15.4%)	1193 (22.8%)
Systolic blood pressure, mmHg	138 ±22	140 ±21
Diastolic blood pressure, mmHg	77 ±12	83 ±11
Antihypertensive medication	3632 (29.6%)	1713 (32.7%)
Diabetes	1125 (9.4%)	610 (12.0%)
Body-mass index	26.9 ±4.1	27.4 ±4.1
Serum cholesterol	6.2 ±1.2	5.5 ±1.1
Serum high-density lipoprotein cholesterol	1.4 ±0.4	1.4 ±0.4
Lipid-lowering medication	1252 (10.2%)	1307 (25.0%)
Smoking		
Former	5299 (44.0%)	2549 (48.6%)
Current	2584 (21.5%)	1082 (20.6%)
Alcohol intake, grams/day (median, IQR)	4.7 (0.4-14.3)	6.0 (0.5-8.6)
Dietary intake*	6.7 ±1.9	n/a
Iron intake, mg/day	12.3 ±3.6	12.8 ±4.1
Glomerular filtration rate, mL/min/1.73m^2	80.0 ±16.0	n/a
C-reactive protein (median, IQR)	1.5 (0.7-3.2)	n/a
Anti-anaemic medication	230 (1.9%)	130 (2.5%)
Vitamin supplements	1967 (16.0%)	1217 (23.3%)
Hormone replacement therapy	361 (2.9%)	142 (2.7%)
APOE genotype		
ε3/ε3	6917 (58.7%)	2938 (58.6%)
ε2/ε2, or ε2/ε3	1568 (13.3%)	653 (13.0%)
ε2/ε4, ε3/ε4, or ε4/ε4	3295 (28.0%)	1420 (28.4%)
Haemoglobin, mmol/L	8.8 ±0.8	8.8 ±0.8
g/dL	14.1 ±1.3	14.1 ±1.2
Mean corpuscular volume, fL/cell	88.9 ±4.9	91.2 ±4.7

Table 1. Baseline characteristics. Data are presented as frequency (%) for categorical, and mean±standard deviation for continuous variables, unless indicated otherwise. IQR=interquartile range; n/a=not available at time of scan. *as measured by adherence to the Dutch dietary guidelines[21]

Overall, 745/12,305 (6.1%) participants had anaemia. The prevalence of anaemia steeply increased with age in men, from 1.4% below 50 years to 33.3% over age 90, whereas in women prevalence was higher at premenopausal ages, yet somewhat lower than in men among the elderly (Figure 1). Of participants with anaemia, 104 had microcytic and 68 had macrocytic anaemia.

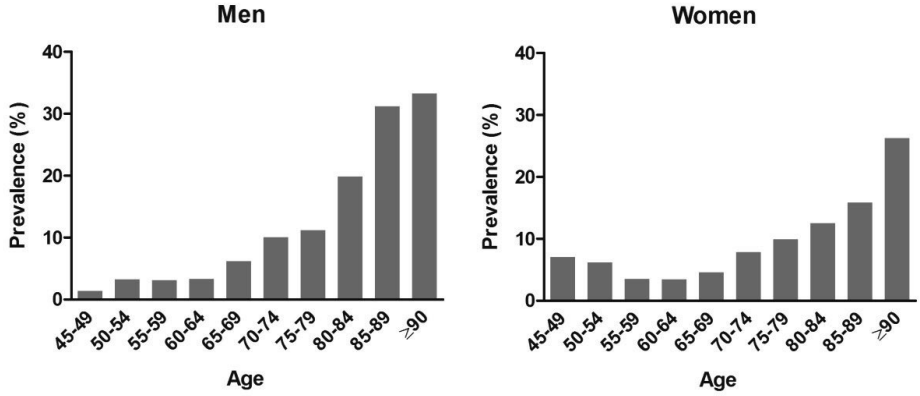

Figure 1. Age-specific prevalence of anaemia

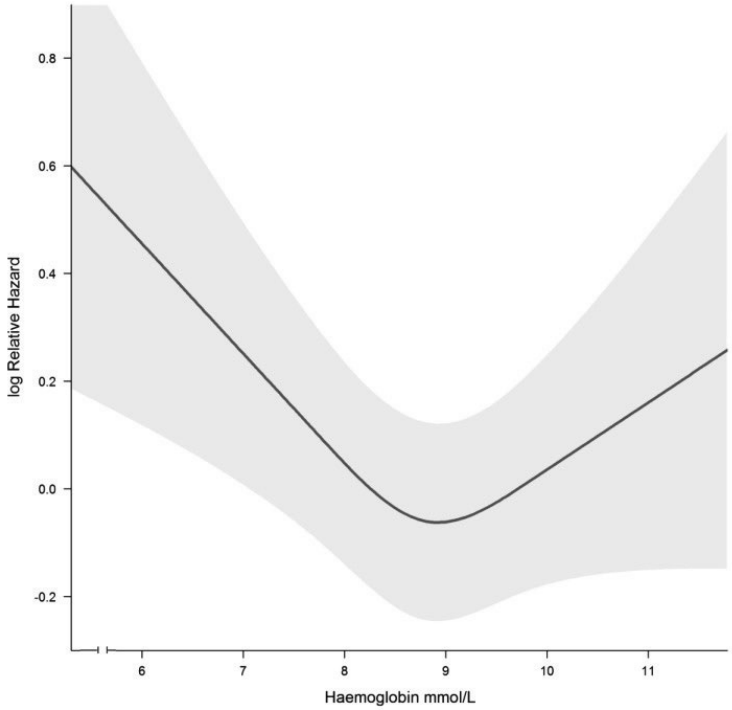

Figure 2. Haemoglobin concentrations and risk of dementia. The non-linearity in the association between haemoglobin and dementia is clearly visible. Shaded areas reflect the 95% confidence limits.

During a mean follow-up time of 12.1 years, 1,520 individuals developed dementia, of whom 1194 (79%) had clinical Alzheimer's disease. Of all incident dementia cases, 222 were preceded by a stroke, a median 4.3 years (IQR 1.4-8.0) before diagnosis of dementia.

Haemoglobin levels at baseline showed a quadratic association with risk of dementia (*P*-value for non-linearity = 0.005; Figure 2), such that risk was increased with both low and high haemoglobin, compared to mid-range levels (Table 2). Risk estimates were slightly higher for Alzheimer's disease only (Table 2), and unaffected by censoring participants at time of stroke (data not shown). Anaemia was associated with a 34% increase in the risk of all-cause dementia, and 41% increase for Alzheimer's disease, with risk estimates highest for macrocytic anaemia, followed by microcytic and normocytic anaemia, respectively (Table 2). Effect estimates were similar upon exclusion of the first 5 years of follow-up (data not shown). The increased risk of dementia with high haemoglobin persisted after excluding individuals with potential causes of secondary erythrocytosis (hazard ratio [95% confidence interval] for highest versus middle quintile: 1.22 [1.02-1.47]). An unmeasured confounder would require an association with both anaemia and dementia by a risk ratio of 2.0 each, above and beyond the measured confounders, to explain away the observed association between anaemia and dementia; for the lower confidence limit to include the null this number is 1.5.

	All-cause dementia (n/N=1,520/12,305)		**Alzheimer's disease (n/N=1,194/12,305)**	
	Model I HR (95% CI)	Model II HR (95% CI)	Model I HR (95% CI)	Model II HR (95% CI)
Haemoglobin at baseline				
1st quintile <8.11 mmol/L	1.29 (1.09-1.53)	1.29 (1.09-1.52)	1.36 (1.13-1.64)	1.36 (1.13-1.64)
2nd quintile 8.11-8.56 mmol/L	1.14 (0.97-1.34)	1.14 (0.97-1.34)	1.21 (1.01-1.45)	1.20 (1.00-1.44)
3rd quintile 8.57-8.99 mmol/L	REFERENCE	REFERENCE	REFERENCE	REFERENCE
4th quintile 9.00-9.40 mmol/L	1.16 (0.98-1.38)	1.17 (0.99-1.39)	1.13 (0.93-1.38)	1.15 (0.94-1.40)
5th quintile >9.40 mmol/L	1.16 (0.97-1.39)	1.20 (1.00-1.44)	1.17 (0.95-1.43)	1.22 (0.99-1.51)
Anaemia*, yes versus no	1.37 (1.14-1.64)	1.34 (1.11-1.62)	1.45 (1.19-1.77)	1.41 (1.15-1.74)
Normocytic†	1.25 (1.02-1.54)	1.22 (0.98-1.51)	1.34 (1.06-1.68)	1.29 (1.02-1.63)
Microcytic†	1.63 (0.98-2.72)	1.75 (1.05-2.93)	1.70 (0.96-3.00)	1.81 (1.01-3.22)
Macrocytic†	2.30 (1.40-3.77)	2.10 (1.25-3.52)	2.45 (1.41-4.25)	2.27 (1.27-4.04)

Table 2. Haemoglobin and anaemia in relation to dementia risk. Model I is adjusted for study cohort, age, and sex; model II is additionally adjusted for educational attainment, smoking habits, alcohol consumption, systolic and diastolic blood pressure, antihypertensive medication, diabetes, serum cholesterol and high-density lipoprotein, lipid-lowering medication, renal function, C-reactive protein, dietary intake including iron, vitamin supplements, anti-anaemic medication, hormone replacement therapy, and *APOE* genotype. *for men: Hb<8.06mmol/L (13.0g/dL); and for women: Hb<7.45mmol/L (12.0g/dL); †Mean corpuscular volume within the normal range (80-95fL) for normocytic, <80fL for microcytic, and >95fL for macrocytic anaemia. HR=hazard ratio; CI=confidence interval.

Of 5,319 participants of the original cohort who underwent brain MRI, 5,281 concurrently had haemoglobin levels measured. Fourteen participants who had dementia at time of MRI were excluded. Compared to the cohort at baseline, individuals who underwent imaging were on average more highly educated, and more often used lipid lowering medication, but had similar haemoglobin levels (Table 1). The association between haemoglobin levels concurrent with MRI, and subsequent risk of dementia was similar to the presented results for the entire cohort (data not shown).

Similar to dementia risk, volume of white matter hyperintensities and structural connectivity (in particular for mean diffusivity) were worse in both the lower and upper ranges of haemoglobin levels (Figure 3). Mean diffusivity was also higher, indicating worse structural connectivity, in individuals with anaemia than in those without (mean difference [95% CI]: 0.207 [0.114-0.301]). These patterns were similar for fractional anisotropy, although risk estimates were somewhat attenuated in the upper range of haemoglobin (Figure 3). Significant associations of low haemoglobin with structural connectivity persisted after additional adjustment for white matter hyperintensity volume (Table 3).

Cerebral microbleeds were present in 1,019 (19.3%) participants (≥2 in 419 [8.0%]), lacunar infarcts in 402 (7.6%), and cortical infarcts in 182 (3.5%). Low levels of haemoglobin, but not high levels, were associated with higher prevalence of microbleeds, such that individuals with anaemia had a 45% higher likelihood of having at least one microbleed (Table 4). The odds ratio (95% CI) increased from 1.29 (0.90-1.86) for harbouring 1 microbleed to 1.59 (1.10-2.31) for ≥2 microbleeds. Haemoglobin levels nor anaemia were related to the presence of lacunar or cortical infarcts (Table 4).

	Microbleeds OR (95% CI)	**Lacunar infarcts** OR (95% CI)	**Cortical infarcts** OR (95% CI)
Haemoglobin at time of MRI			
1st quintile <8.11 mmol/L (<13.07 g/dL)	1.22 (0.97-1.52)	0.90 (0.64-1.28)	0.75 (0.47-1.21)
2nd quintile 8.11-8.56 mmol/L (13.07-13.79 g/dL)	1.02 (0.81-1.28)	1.09 (0.78-1.53)	0.82 (0.51-1.32)
3rd quintile 8.57-8.99 mmol/L (13.80-14.40 g/dL)	REFERENCE	REFERENCE	REFERENCE
4th quintile 9.00-9.39 mmol/L (14.41-15.17 g/dL)	0.93 (0.74-1.18)	1.09 (0.78-1.54)	0.62 (0.37-1.02)
5th quintile >9.39 mmol/L (>15.17 g/dL)	0.92 (0.72-1.17)	1.02 (0.72-1.44)	0.86 (0.54-1.36)
Anaemia*, yes versus no (n=285/5267)	1.45 (1.09-1.93)	1.02 (0.67-1.54)	0.83 (0.44-1.55)

Table 4. Haemoglobin and anaemia in relation to focal imaging markers of vascular brain disease. The model is adjusted for age, sex, total intracranial volume, educational attainment, smoking habits, alcohol consumption, systolic and diastolic blood pressure, antihypertensive medication, diabetes, serum cholesterol and high-density lipoprotein, lipid-lowering medication, vitamin supplements, anti-anaemic medication, *APOE* genotype, and interval between blood sampling and MRI. *for men: Hb<8.06mmol/L (13.0g/dL); and for women: Hb<7.45mmol/L (12.0g/dL). OR=odds ratio; CI=confidence interval.

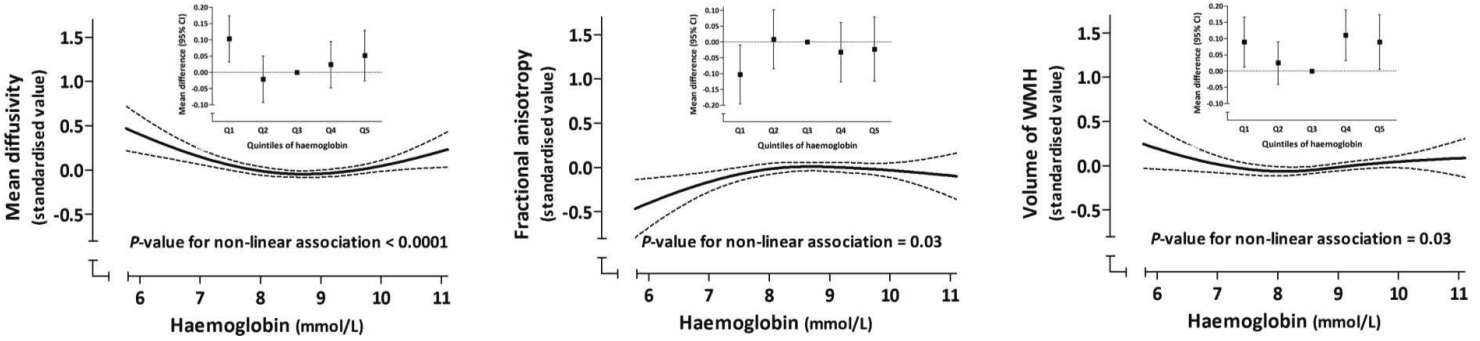

Figure 3. Haemoglobin in relation to white matter integrity and volume of WMH. White matter integrity is represented by mean diffusivity (left; higher reflects worse integrity), fractional anisotropy (middle; lower reflects worse integrity), and volume of white matter hyperintensities (right). WMH=white matter hyperintensities. Solid black lines represent the association of haemoglobin levels with mean diffusivity (left), fractional anisotropy (middle), and volume of WMH (right). Dotted lines reflect the 95% confidence interval. The inline figures show values per quintile of haemoglobin, as compared to the middle (reference) quintile.

	Mean diffusivity β (95% CI)	Fractional anisotropy β (95% CI)	WMH β (95% CI)	Mean diffusivity† β (95% CI)	Fractional anisotropy† β (95% CI)
Haemoglobin at time of MRI					
1st quintile (n=1071)	0.103 (0.032;0.174)	-0.103 (-0.196;-0.010)	0.089 (0.012;0.166)	0.071 (0.006;0.136)	-0.063 (-0.149;0.023)
2nd quintile (n=1019)	-0.021 (-0.093;0.050)	0.008 (-0.085;0.102)	0.025 (-0.041;0.090)	-0.030 (-0.096;0.035)	0.020 (-0.066;0.106)
3rd quintile (n=1096)	REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE
4th quintile (n=945)	0.024 (-0.048;0.095)	-0.032 (-0.126;0.062)	0.110 (0.032;0.188)	-0.016 (-0.082;0.050)	0.018 (-0.069;0.105)
5th quintile (n=1136)	0.052 (-0.026;0.129)	-0.023 (-0.124;0.079)	0.089 (0.005;0.173)	0.020 (-0.051;0.091)	0.017 (-0.077;0.111)
Anaemia* (284/5267)	0.207 (0.114;0.301)	-0.172 (-0.294;-0.050)	0.036 (-0.065;0.138)	0.194 (0.108;0.280)	-0.156 (-0.269;-0.042)

Table 3. Haemoglobin in relation to white matter integrity, with and without adjustment for white matter hyperintensity volume. Values express change in standardised measures of MD, FA, and WMH. Higher values of MD and lower values of FA reflect worse structural integrity. The model is adjusted for age, sex, total intracranial volume, educational attainment, cardiovascular risk factors, vitamin supplements, anti-anaemic medication, *APOE* genotype. †additionally adjusted for volume of white matter hyperintensities; *for men: Hb<8.06mmol/L (13.0g/dL); and women: Hb<7.45mmol/L (12.0g/dL). WMH=white matter hyperintensities.

Finally, haemoglobin levels were linearly associated with cerebral perfusion (Figure 4), such that 1 mmol/L increase in haemoglobin was associated with 3.2 mL/100mL/min lower cerebral perfusion (95% CI: 2.8-3.6, *P*<0.0001). This association was most profound in individuals with higher volumes of WMH (Figure 3; P-value for interaction = 0.002), with effect estimates increasing from 2.5 (1.7-3.2) mL/100mL/min in the lowest tertile to 4.0 (3.3-4.6) mL/100mL/min in the highest tertile of WMH. Blood pressure levels did not modify the association between haemoglobin and cerebral perfusion (mean arterial pressure: *P*= 0.71).

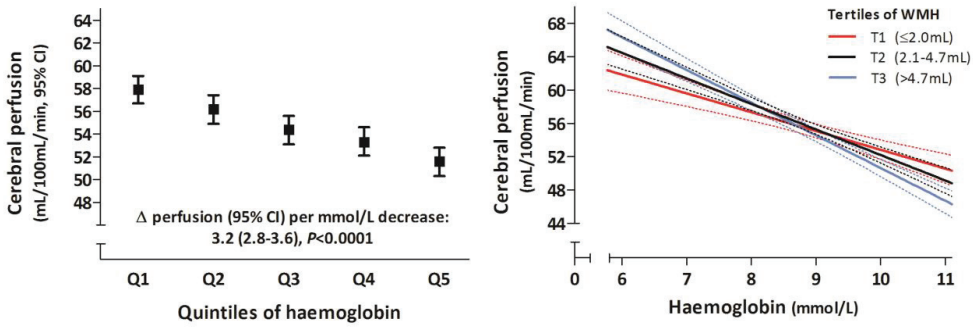

Figure 4. Haemoglobin and cerebral perfusion. Cerebral perfusion is presented per quintile of haemoglobin (left), and continuously, stratified by severity of white matter hyperintensities on MRI (right; upper and lower limits for stratification in tertiles are shown in the figure legend).

DISCUSSION

In this large population-based study, we found that both low and high levels of haemoglobin are associated with an increase in the long-term risk of dementia, including Alzheimer's disease. Anaemia relates to a 34% increase in dementia risk, and 41% for Alzheimer's disease. Brain imaging findings suggest white matter structural connectivity, cerebral perfusion, and potentially microbleeds as pathophysiological substrates in these associations.

The prevalence of anaemia in our study of 6%, and its steep increase with age to roughly 30% in the very elderly, are similar to what has been reported in other European and North-American populations.[3,7,8,12-14] The worldwide prevalence of anaemia, however, is much higher, and the regions most affected mirror those with the steepest increases in the incidence of non-communicable diseases including dementia.[1,9] In line with three prior reports,[12-14] we show that this may have implications for the burden of dementia, as long-term associations with anaemia extend to the low-normal range of haemoglobin. The central

question that remains, of course, is to which extent haemoglobin levels are directly accountable for this increased risk (e.g. by reduced tissue oxygenation), or whether the associations can be explained by underlying or concomitant vascular or metabolic changes, notably involving iron, and vitamins B9 (folate) and B12.[26-28] Anaemia can coincide with a wide range of (chronic) conditions, some of which are very rare in the community (e.g. myelodysplastic syndrome, thalassemia), while others (e.g. iron deficiency and inflammation) are common, and could contribute to cognitive decline via pathways unrelated to haemoglobin. We controlled for various such (sub)clinical disease measures in our analyses, including C-reactive protein, and the calculated E-value shows that substantial confounding by other variables would be required to explain away the associations. Nevertheless, potential pathways, also including unmeasured factors like iron, warrant consideration.

As for direct effects, reduced oxygenation can lead to hypoxia and subsequent inflammation with deleterious effects on neurons. The tight correlation of haemoglobin to cerebral blood flow which we found supports the notion of a compensatory mechanism that maintains cerebral oxygen delivery,[29] and may be crucial in case of impaired oxygen extraction and failing autoregulatory mechanisms with cerebral small-vessel disease.[25,30] Iron chelators are thought to maintain levels of hypoxia inducible factor 1-alpha (HIF-1α) in the nerve, potentially linking haemoglobin to poor oxygenation and Alzheimer's disease.[31] When oxygenation falls below the threshold for ischaemia, anaemia might even lead to ischaemic stroke.[5,32] Yet, clinical stroke did not account for associations in our study, and the absence of an associations between low haemoglobin and infarcts on brain MRI, in line with another imaging study,[33] suggests that more subtle, chronic processes are involved in cognitive decline. This is illustrated by the increased burden of white matter hyperintensities and reduced structural connectivity with low and high haemoglobin levels, mirroring the observed dementia risk increase. Although diffusion imaging is unable to differentiate between axonal loss and demyelination, these findings could point to lower or upper range disturbance of iron homeostasis. Iron is vital for various cellular processes in the brain, including neurotransmitter synthesis, mitochondrial function, and myelination of neurons.[34] Further study assessing plasma iron, but also transferrin and folate,[35] in relation to dementia and biomarkers of Alzheimer's disease[28,36] may therefore unravel these associations further.

In addition to the lower range, we found that high haemoglobin levels were also associated with an increased risk of dementia, extending short-term observations in a prior study to long-term risk in the community.[13] Although studies often focus on anaemia and the lower range of haemoglobin levels, high haemoglobin may be deleterious, or reflect deleterious circumstances, in several ways. First, lysis of erythrocytes could cause elevated

measurements in the presence of functional anaemia and excess of free iron. Second, erythrocytosis occurs secondary to systemic reductions in blood oxygenation, often due to smoking, heart failure, chronic obstructive pulmonary disease, or chronic kidney disease.[37] These are all risk factors for dementia, yet excluding participants with common causes of erythrocytosis from our analysis did not attenuate the risk estimates for high haemoglobin. Third, higher blood viscosity could predispose to ischaemia, as typically seen in patients with polycythemia vera.[38] Elevated haematocrit values have been observed in patients with TIA and stroke[39] and covert brain infarcts,[40] and although we found no association of high haemoglobin with infarcts on MRI, the previously reported U-shaped associations of haematocrit with risk of dementia, as well as coronary heart disease and ischaemic stroke during long-term follow-up corroborate the relevance of deviations on both sides from the normal range.[5] Further studies are needed to determine whether these findings are for instance attributable to high viscosity, reductions in tissue oxygenation with very high levels of haematocrit,[41] or the higher haematocrit levels in individuals with microangiopathy.[42,43]

Several limitations to our study need to be taken into account. First, despite robustness of the reported associations to adjustment for a wide range of potential confounders, including (sub)clinical measures of chronic disease, residual confounding may hamper causal inference. In particular, we did not measure (transport of) iron and B vitamins, and variations in their levels and metabolism may contribute to neurodegeneration. However, as demonstrated by the E-value of 2.0, such confounding would need to have strong associations with both determinant and outcome. Second, the subset of our population undergoing MRI was more highly educated and more often used lipid-lowering medication than the cohort at baseline, although with similar haemoglobin levels in both groups this most likely will not have led to bias in the relative risks. Third, our Dutch population is predominantly of European descent, and further study is needed to confirm that these findings indeed apply to other ethnicities and geographic regions. For example, mutations at the alpha thalassemia gene, which are common in African descent, and varying prevalence of malaria and sickle cells might contribute to (complications of) anaemia differently from what we observe in our population.[44]

In conclusion, both low and high levels of haemoglobin are associated with an increased long-term risk of dementia among the general population. Given the potential implications for the burden of dementia globally, studies are needed to identify biological substrates, potentially focussing around disturbances in structural brain connectivity and cerebral blood flow regulation.

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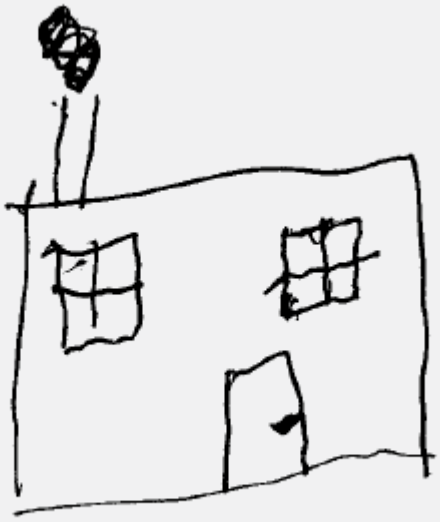
Chapter 3.5

Carotid artery stenosis



ABSTRACT

Low cerebral perfusion is a risk factor for developing dementia, and may represent a novel target for intervention against cognitive decline. Carotid artery stenosis is a common cause of haemodynamic impairment of cerebral blood flow, but its association with neurodegeneration remains uncertain. We compared hemispheres of community-dwelling individuals with unilateral carotid artery stenosis to investigate neurodegeneration in relation to haemodynamic impairment of brain perfusion. From consecutive participants of the population-based Rotterdam Study who underwent structural brain magnetic resonance (MR) imaging and time-of-flight MR angiography, we selected all participants with a unilateral ≥50% stenosis at the carotid artery bifurcation, and <30% stenosis on the contralateral side (NASCET criteria). Brain tissue volumes were extracted using an automated tissue segmentation method, and cerebral blood flow through the carotids was measured by 2D phase-contrast MR imaging. Among 50 eligible participants (mean age 76 years, 50% women), median grade of stenosis on the affected side was 57% (IQR 51-65%). Flow was on average lower in the affected carotid artery (160 mL/min versus 202 mL/min on the unaffected side; *P*=0.0002), with larger reductions in flow with higher degree of stenosis (reduction in flow [95% CI] per 1% increase in stenosis = 1.7 mL/min [1.0-2.5], *P*<0.0001). Downstream of the stenotic artery, parenchymal volume was smaller than in the contralateral hemisphere (mean difference [95% confidence interval] -2.7 mL [-4.9;-0.4]), similar for grey and white matter, and unaffected by excluding individuals with prior TIA or stroke. Differences were most profound in the frontoparietal lobes, and increased with severity of stenosis to roughly 5 mL in individuals with the haemodynamically most significant stenosis (≥70%). White matter hyperintensity volume and microstructural integrity did not differ between hemispheres. In conclusion, carotid artery stenosis is associated with parenchymal tissue loss, with effects equivalent to 1-2 years of brain ageing.

INTRODUCTION

Reduced cerebral blood flow and impairment of flow regulation are associated with an increased risk of developing dementia,[1,2] which could open new avenues for preventive interventions against cognitive decline. Stenosis of the major brain supplying arteries is a common cause of disturbed blood flow, and stenosis of the carotid artery bifurcation in particular has had much attention for its high risk of early recurrent stroke, and clear benefits of surgical intervention.[3,4] A limited number of studies, however, have investigated stenosis of the internal carotid artery (ICA) in relation to cognition and dementia. In cross-sectional studies, extracranial ICA stenosis has been associated with prevalence of dementia,[5] and worse cognitive function in non-demented individuals,[6-8] extending to cognitive decline during follow-up in one of these studies.[8] The notion that hypoperfusion may be the underlying mechanism is strengthened by observations that stenosis is particularly detrimental when accompanied by impaired cerebrovascular reactivity.[9,10] Yet, studies comparing patients with stenosis to controls remain highly susceptible to (residual) confounding, as ICA stenosis shares many risk factors with dementia that invariably contribute to cognitive decline via pathways other than stenotic disease. This confounding may be overcome by the comparison of brain regions downstream of a unilateral haemodynamically significant stenosis with the equivalent regions in the other, unexposed hemisphere of the same individual.

Nested within a prospective population-based cohort, we therefore aimed to determine inter-hemispheric differences in markers of neurodegeneration in individuals with unilateral carotid artery bifurcation stenosis.

METHODS

Study population

This study is embedded within the Rotterdam Study, a large population-based cohort study in the Netherlands.[11] The study population consists of 14,926 individuals aged ≥45 years from the Ommoord area, a suburb of Rotterdam, who undergo extensive routine examinations at four-yearly visits to a dedicated research facility. At each centre visit, participants undergo structural brain magnetic resonance imaging (MRI), and are screened for subclinical atherosclerosis by ultrasound measurement of the carotid artery intima-media thickness.[12] From October 2007 onwards, all individuals with an intima-media thickness >2.0mm in either carotid artery were invited for time-of-flight magnetic resonance angiography (MRA) to assess carotid artery bifurcation stenosis.[13] Of invitees, 1,982 (74%)

underwent MRA. Internal carotid artery (ICA) stenosis at the bifurcation was present in 238 (12%) individuals, of whom 110 had no stenosis in the contralateral ICA. Of these 110 individuals, 53 with structural brain MRI within 6 months of arterial imaging were considered eligible for the current study.

Time-of-flight MRA

Magnetic resonance imaging of the carotid arteries was performed on a 1.5T MR scanner (General Electric Healthcare, Milwaukee, WI, USA) with a bilateral phased-array surface coil (Machnet, Eelde, The Netherlands).[13] The position of the participants was stabilised in a custom-designed head holder to reduce motion artefacts. High-resolution images were obtained using a standardized protocol. First, both carotid bifurcations were identified by means of two-dimensional time-of-flight MRA. Next, high-resolution MRI sequences captured the carotid bifurcations on both sides, as described in detail previously.[13] All images were assessed for the presence of stenosis by a trained physician under the supervision of an experienced neuroradiologist, blinded to the clinical status, and the severity of stenosis was graded according to NASCET criteria.[14]

Brain MRI scan protocol and image processing

MRI of the brain was performed on a 1.5T scanner (General Electric Healthcare, Milwaukee, WI, USA), using an 8-channel head coil.[15] We acquired high-resolution axial T1-weighted sequence, proton-density-weighted (PD) sequence, and fluid attenuated inversion recovery (FLAIR) sequence. For flow measurement, 2D phase-contrast imaging was performed as described previously.[16] In brief, a sagittal 2D phase-contrast angiographic scout image was performed. On this scout image, a transverse imaging plane perpendicular to both the pre-cavernous portion of the internal carotid arteries and the middle part of the basilar artery was chosen for a 2D gradient-echo phase-contrast sequence (repetition time=20 ms, echo time=4 ms, field of view=19 cm2, matrix=256 × 160, flip angle=8°, number of excitations=8, bandwidth=22.73 kHz, velocity encoding=120 cm/sec, slice thickness=5 mm). Acquisition time was 51 seconds and no cardiac gating was performed. For diffusion-MRI, we performed a single shot, diffusion-weighted spin echo echo-planar imaging sequence. Maximum b-value was 1000 s/mm2 in 25 non-collinear directions; three volumes were acquired without diffusion weighting (b-value = 0 s/mm2).[17]

Flow was calculated from the phase-contrast images using interactive data language-based custom software (Cinetool version 4; General Electric Healthcare). Two independent, experienced technicians manually drew the regions of interest (i.e. surface area of the left and right carotid artery, and basilar artery) in a plane perpendicular to the arteries at the level of the carotid clinoid segment, and performed subsequent flow measurements per

vessel (mL/min; inter-rater correlations >0.94 for all vessels).[16] For the assessment of brain volume, the structural MR sequences (T1-weighted, PD-weighted, and FLAIR) were transferred to a Linux workstation. Pre-processing steps and the classification algorithm have been described previously.(18) Quantification of cerebrospinal fluid, total parenchymal volume, and white matter hyperintensity (WHM) volume were done using an automated tissue segmentation method, based on a k-nearest-neighbour brain tissue classifier algorithm.[18] All segmentation results were visually inspected and if needed manually corrected by investigators who were blinded to the carotid artery stenosis measurements. Parenchymal brain volume was calculated by adding up grey and white matter volumes, converted to millilitres. All diffusion data were pre-processed using a standardized pipeline,[17] fitting diffusion tensors to compute mean fractional anisotropy (FA) and mean diffusivity (MD) in the normal-appearing white matter through combination with the tissue segmentation. In general, lower FA and higher MD values are considered indicative of lower microstructural integrity. All scans were furthermore inspected by trained research physicians, blinded to stenosis site, for the presence of cortical infarction.

Other measurements

Medical history and information on cardiovascular risk factors was obtained during study centre visits on the basis of measurement assessed by interview, with verification in medical records if appropriate.

Analysis

Among all eligible participants, we first determined to which extent flow was reduced downstream of the ICA stenosis, compared to the contralateral side using a paired t-test, and subsequently assessed whether degree of stenosis in the stenotic (≥50%) artery was related to blood flow using linear regression.

We then performed a paired t-test to determine interhemispheric differences in total parenchymal volume in relation to the presence of ICA stenosis, before and after exclusion of participants with radiographic evidence of cortical infarction, or a clinical history of TIA or stroke. For reliability of tissue segmentations, and generalisability to individuals without (covert) brain infarcts, we excluded individuals with cortical infarction from the subsequent analyses. We tested for interhemispheric differences, segregated by grey and white matter, and stratified results by brain region. Given the arterial territory supplied by the ICA, we expected the largest interhemispheric differences in the frontoparietal lobes. We repeated these analyses for increasing severity of stenosis (i.e. 50-59%, 60-69%, and ≥70%), and for increasing reduction in downstream blood flow (i.e. flow through the affected ICA as a percentage of flow through the unaffected ICA). We furthermore assessed whether

associations differed by systemic mean arterial pressure, age at time of brain imaging, and side of the stenosis. Parenchymal tissue comparisons were repeated using volumetric segmentations acquired with the FreeSurfer image analysis suite (version 6.0.0), which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). Finally, we performed an interhemispheric comparison of the burden of WMH, and mean diffusivity and fractional anisotropy as measures of microstructural white matter integrity.

Analyses were done using IBM SPSS Statistics version 23.0 (IBM Corp, Armonk, NY, USA). Alpha-level (type 1 error) was set at 0.05.

RESULTS

Of 53 eligible participants, 2 were excluded because of large meningioma, and 1 because of movement artefact on brain imaging, leaving 50 participants for analysis. Characteristics of the study population are shown in Table 1. A stenosis of ≥50% was present in the left ICA in 24 (48.0%) participants, and right-sided in 26 (52.0%) participants. Flow through the ICA was lower downstream of the stenosis than on the contralateral side (160 mL/min versus 202 mL/min, *P*=0.0002), and the degree of stenosis was related to the degree of flow reduction (reduction in flow [95% CI] per 1% increase in stenosis = 1.7 mL/min [1.0-2.5], *P*<0.0001).

Characteristics	Study population
Age, years	75.8 (±7.6)
Female sex	25 (50.0%)
Smoking	
Former	33 (66.0%)
Current	10 (20.0%)
Systolic blood pressure, mmHg	150 (±21)
Diastolic blood pressure, mmHg	82 (±10)
Blood pressure lowering medication	30 (60.0%)
Total cholesterol, mmol/L	5.4 (±1.3)
High density lipoprotein cholesterol, mmol/L	1.4 (±0.4)
Lipid lowering medication	23 (46.0%)
Diabetes	13 (28.9%)
Body-mass index, kg/m^2	27.1 (±3.6)
History of TIA or stroke	10 (20.0%)
Antithrombotic medication	27 (54.0%)
Carotid artery stenosis, % (median, IQR)	57 (51-65)
50-59%	28 (56.0%)
60-69%	14 (28.0%)
≥70%	8 (16.0%)
Contralateral stenosis, % (median, IQR)	11 (0-23)

Table 1. Baseline characteristics of the 50 participants. Values are depicted as mean±standard deviation for continuous variables, and absolute numbers (%) for categorical variables, unless indicated otherwise. TIA=transient ischaemic attack.

Four individuals had radiographic evidence of cortical infarction, all on the side of the stenosis, and ten participants had reported (transient) neurological symptoms consistent with ischaemia. Overall, parenchymal volume was lower on the side of the stenosis than on the contralateral side (mean difference [95% CI] -3.2 mL [-5.4;-1.0]). This difference slightly attenuated after excluding the four individuals with evidence of cortical infarction on MRI (-2.7 mL [-4.9;-0.4]), but was unaffected by further exclusion of participants with clinically overt cerebrovascular disease (-2.7 mL [-5.2;-0.2]).

Proceeding with region-specific analyses among individuals without cortical infarction on MRI, interhemispheric differences in tissue volumes were most profound in the frontal and parietal lobes, and were similar for grey and white matter tissue (Table 2). Interhemispheric differences increased both with degree of stenosis, and with percentage of flow reduction, to a roughly 5 mL difference in individuals with the haemodynamically most significant stenosis (Table 3).

	Parenchymal volume Δ mL (95% CI)	**Share*** (%)	**Grey matter** Δ mL (95% CI)	**Share*** (%)	**White Matter‡** Δ mL (95% CI)	**Share*** (%)
Hemisphere	-2.7 (-4.9;-0.4)	-0.62	-1.6 (-3.3;0.2)	-0.64	-0.9 (-1.8;0.1)	-0.50
Frontal lobe	-1.5 (-2.4;-0.6)	-1.02	-0.7 (-1.2;-0.1)	-0.85	-0.8 (-1.3;-0.2)	-1.24
Temporal lobe	-0.6 (-2.6;1.4)	-0.66	-0.3 (-1.6;1.1)	-0.51	-0.3 (-1.0;0.3)	-0.92
Parietal lobe	-1.0 (-2.4;0.4)	-1.10	-0.7 (-1.4;0.1)	-1.42	-0.2 (-1.0;0.6)	-0.49
Occipital lobe	0.4 (-0.5;1.3)	+0.74	-0.001 (-0.6;0.6)	-0.003	0.4 (-0.04;0.8)	+1.89

Table 2. Interhemispheric difference in parenchymal tissue volume. Numbers reflect the difference between affected and unaffected hemisphere, i.e. a negative sign means volumes were lower on the affected side.
Δ mL=interhemispheric difference in millilitres. * Percentage of mean hemispheric volume; ‡ Normal-appearing white matter; CI=confidence interval.

Differences In parenchymal volume were most prominent in older participants (≥75 years -5.5 [-8.4;-2.6] versus 0.1 [-3.0;3.3] <75 years), and in those with left-sided stenosis (-7.3 mL [-9.9;-4.8] for left-sided versus 0.6 mL [-2.3;3.6] for right-sided stenosis), despite a similar degree of luminal narrowing on both sides (median [IQR] for left– 57% [51-69] versus right– 58% [54-63], *P*=0.84). Associations did not meaningfully differ with levels of mean arterial pressure (data not shown). The use of FreeSurfer brain tissue segmentations yielded similar results (overall mean difference -3.1 mL [-5.2;-1.1]).

We observed no interhemispheric differences in WMH volume (2.8 mL [1.7% of white matter volume] on the side of stenosis, versus 3.0 mL [1.8%] on the contralateral side; *P*=0.23). This was similar on regions-specific analyses per lobe (data not shown). Microstructural white matter integrity was also similar between hemispheres (standardised difference [95% CI] in mean diffusivity -0.002 [-0.097;0.093], and fractional anisotropy 0.004 [-0.100;0.108]).

	*N**	**Parenchymal volume** Difference in mL (95% CI)
Stenosis degree		
50-59%	25	-1.4 (-4.6;1.8)
60-69%	13	-3.5 (-8.3;1.3)
≥70%	8	-5.1 (-10.9;0.7)
Flow reduction		
No flow reduction	10	0.3 (-3.8;4.4)
1-14%	12	-1.7 (-6.3;3.0)
15-29%	12	-3.1 (-8.7;2.6)
≥30%	12	-5.7 (-10.6;-0.8)

Table 3. Interhemispheric difference by severity of stenosis. * Four individuals with evidence of cortical infarction on MRI were excluded.

DISCUSSION

In this study among community-dwelling individuals with unilateral carotid artery stenosis, we found smaller parenchymal volumes downstream of the stenosis than in the contralateral hemisphere. Differences were larger with increasing severity of stenosis, and most profound in the ICA flow territory. The observed differences are equivalent to about 2 years of cerebral atrophy.[19]

Given its importance in the prevention of recurrent stroke, it is remarkable that no published studies have prospectively assessed the risk of dementia with carotid artery stenosis. In cross-sectional studies, carotid stenosis has been associated with prevalence of dementia,[5] and worse cognitive function in non-demented individuals,[6-8] which coincided with smaller total brain volume in one study,[6] and extended to decline in cognitive test performance during follow-up in another.[8] In the present study, eliminating confounding of concomitant vascular disease by design, we provide evidence on a biomarker level that this variation in clinical function may indeed be due to stenotic disease. In view of potential preventive interventions, this raises the question whether these associations reflect haemodynamic or thromboembolic consequences of stenosis.

Brain infarcts on imaging were present in 4/50 participants in our study, all downstream of the ICA stenosis, but did not explain the observed interhemispheric differences in parenchymal volume. Of two population-based studies in which carotid artery stenosis related to worse cognitive performance, covert brain infarcts were more common in individuals with carotid artery stenosis in one,[6] but not the other.[7] Taken together, this suggests that larger infarcts are contributing, but not solely responsible for the association between carotid artery stenosis and cognitive decline. Yet, thromboembolic complications of

stenosis may be more subtle than what is routinely visualised on MRI. Micro-infarcts have recently emerged as a risk factor for cognitive impairment,[20] and the contribution of micro-emboli, hypoperfusion, and cerebral small-vessel disease to their occurrence renders it conceivable that micro-infarcts occur commonly with stenosis.[20] Small cortical infarcts on brain imaging and micro-infarcts on neuropathology appear predominantly in the watershed areas, supporting a haemodynamic origin.[21,22] In preclinical studies in mice, these micro-infarcts, along with microhaemorrhages, white matter damage, (vascular) amyloid deposition, and neuronal loss and memory impairment,[23-28] are seen with chronic cerebral hypoperfusion, commonly induced by bilateral partial carotid artery coiling. Flow-limiting effects of stenosis on the brain are further corroborated by observations that stenosis is particularly detrimental when accompanied by impaired cerebrovascular reactivity.[9,10] Reduced vascular reactivity likely indicates worse haemodynamic impairment,[29,30] and joint assessment of stenosis and downstream reactivity may thus provide more accurate measures of physiological impact. We did not measure vasoreactivity in the present study, and as the vast majority of micro-infarcts currently remain under the detection limit of in vivo clinical imaging,[31] we were unable to further investigate this on 1.5T imaging.

The lack of association between stenosis and white matter hyperintensities is consistent with four out of five prior studies assessing inter-hemispheric differences.[32] This could be consistent with the hypothesis that effects of ICA stenosis on cognition are mainly due to arterial stiffening rather than luminal narrowing.[33] Although only 14/820 participants in that particular study had luminal narrowing exceeding 50%,[33] precluding firm conclusions about haemodynamic effects, it may well be large artery stiffening and increased downstream pulsatility that give rise to leukoaraiosis.[34,35] Given the often systemic presence of atherosclerosis,[36] such effects would not be detected by assessing inter-hemispheric differences. Nevertheless, it remains to be determined whether hypoperfusion itself contributes to small-vessel disease,[37] in particular as the absence of an association in prior inter-hemispheric comparisons could also be due to differences in design, in terms of inclusion of stenosis degree (>30% to >70%) and outcome measurement (mostly visual inspection rather than volumetric segmentation).[32] Relatively mild stenosis may be insufficient to detect differences in small studies, especially in the absence of a sensitive quantitative outcome measure. In two other studies comparing individuals with and without stenosis, carotid artery stenosis was associated with volume of WMH in one (using automated segmentation),[6] but not another study (using visual rating).[7] Regarding white matter microstructure, a meta-analysis of six small studies suggests changes in FA and the apparent diffusion coefficient (ADC), but not MD, with carotid artery stenosis.[38] We did not find evidence of such an association in the present study.

Remarkably, we found associations mainly for left-sided stenosis. Given the similar associations using FreeSurfer segmentation, these differences are unlikely to be due to systemic error in the tissue segmentations. To rule out a physiological asymmetry that accounts for the observed differences, we assessed interhemispheric differences in a random sample of 1,000 participants of the Rotterdam Study without carotid artery stenosis. In this group we found *right*ward occipital asymmetry, with less profound *left*ward asymmetry in the frontal lobe (Figure 1), similar to what was recently observed in the large ENIGMA consortium.[39] This implies that the smaller frontal and temporal lobe volumes with left-side stenosis in our study are not merely a natural physiological phenomenon, but might even be a mild underestimation of a true interhemispheric difference. Of note, the Cardiovascular Health Study reported associations of high-grade stenosis of the left, but not the right carotid artery with cognitive impairment and cognitive decline.[8] Taken together, this could point to preferential flow of microemboli from concomitant aortic stenosis into the left common carotid artery, higher plaque vulnerability on the left side,[40] or more profound effects of flow impairment on the dominant, metabolically perhaps more active hemisphere. However, numbers of individuals with stenosis in both the Cardiovascular Health Study and the study presented here are small, and lateralisation due to chance may well overrule these rather speculative explanations.

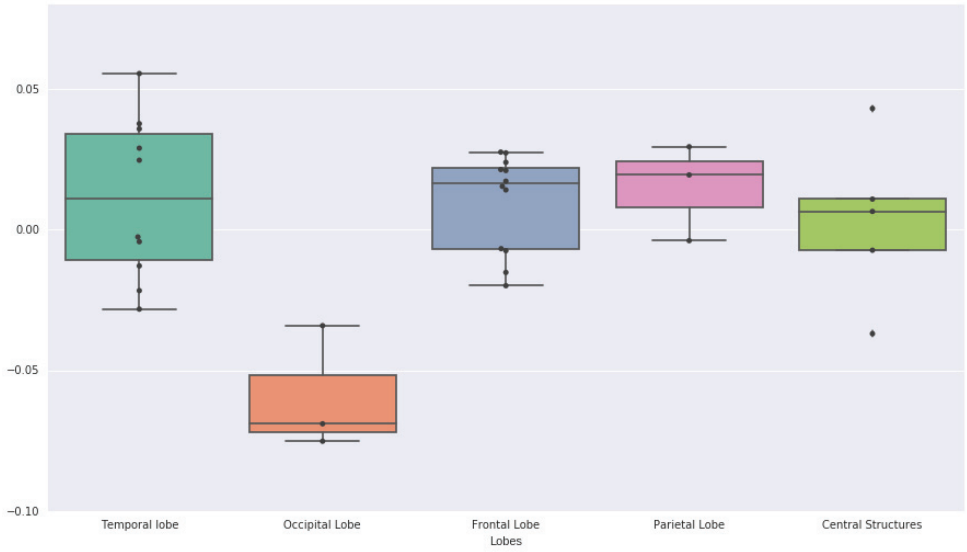


Figure 1. Brain asymmetry in a random sample of 1,000 Rotterdam Study participants without significant carotid artery stenosis, we determined the asymmetry index[53] (AI; vertical axis) for each lobe separately. The AI is a measure of grey matter asymmetry, calculated by voxel-wise comparison of grey matter content on the original and flipped image: $AI = \frac{original\ image - flipped\ image}{0.5 * (original\ image - flipped\ image)}$. AI values are depicted here for the left hemisphere, and a positive AI value consequently indicates more grey matter in the left hemisphere (leftward asymmetry), whereas a negative value indicates rightward asymmetry.

Numerous reports of various methodological rigor have suggested that cognitive performance may be improved after carotid artery desobstruction, whether by stenting or endarterectomy, and for symptomatic as well as asymptomatic stenosis.[41-51] In the absence of randomised controlled trials, these should be no basis for routine intervention, but they support aetiological involvement of haemodynamically significant stenosis of the brain supplying arteries, and advocate for incorporation of cognitive endpoints in intervention trials of carotid stenosis for stroke prevention in the statin era. Whilst for stroke prevention such interventions depend on the symptomatology of the stenotic disease, the prolonged exposure contributing to neurodegeneration and cognitive decline may well pass unnoticed for a long time, and fit a different paradigm. Optimal medical treatment for stenosis may thereby too play an important part in the prevention of cognitive impairment.[52]

Certain limitations of this study need to be taken into account when interpreting the findings. First, structural MRI and MRA were not performed concurrently, and although time differences were limited to six months by design, the progressive nature of arterial stenotic disease may have biased our findings to the null if structural MRI was acquired before MRA. Second, we did not measure stenosis of the cerebropetal vessels beyond the carotid artery bifurcation. Concurrent intracranial stenosis may have caused bias in either direction, whereas vertebrobasilar stenosis would most likely enhance occipital lobe differences. Third, given the nature of the study, we were unable to assess the effect of stenosis on cognitive performance, but the magnitude of effects, equivalent to about 2 years of atrophy, suggests such effects are likely to be clinically meaningful. Finally, the limited sample size hampers precision, in particular for subgroup analyses, and our findings require replication in larger studies also using automated imaging segmentation in patients with symptomatic and asymptomatic stenotic disease.

In conclusion, carotid artery stenosis is associated with cerebral atrophy, but in this relatively small sample not with markers of white matter integrity. Future studies are needed to affirm these findings, disentangle haemodynamic from thromboembolic effects, and assess the effect of medical treatment and revascularisation procedures on markers of neurodegeneration and cognitive performance.

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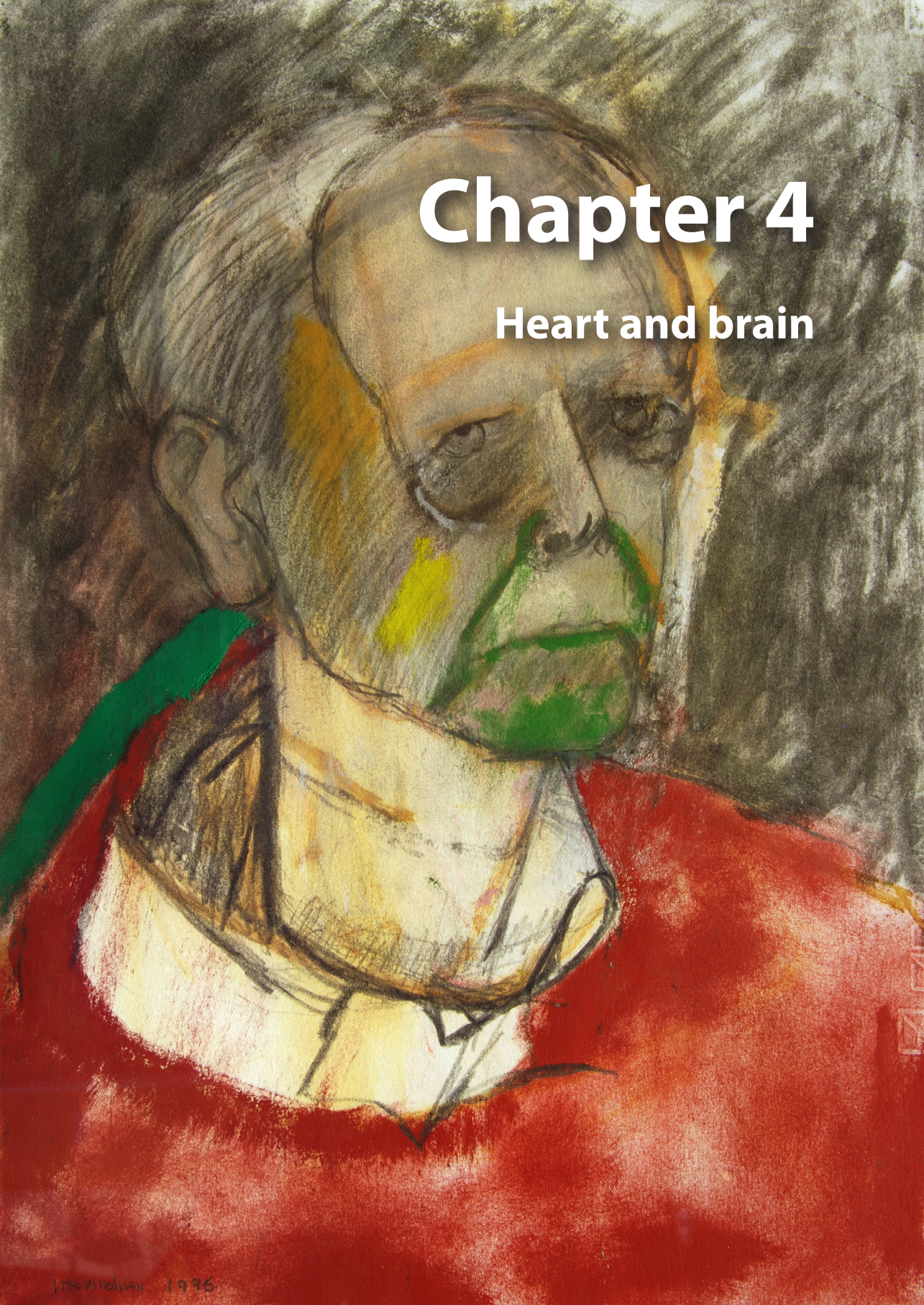
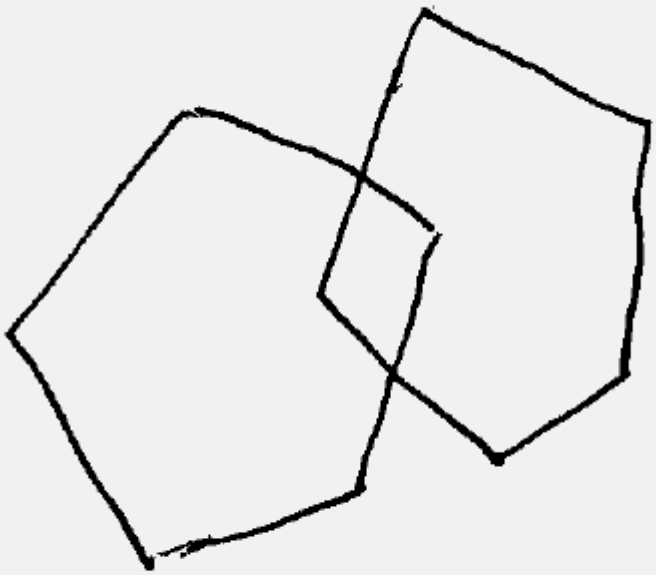


Chapter 4

Heart and brain

Chapter 4.1

Heart disease and dementia



ABSTRACT

With the improved care for patients with myocardial infarction and heart failure, patients live longer with suboptimal cardiac function, and are increasingly susceptible to late-life diseases including dementia. Cardiovascular risk factors are closely linked with future dementia risk, but whether heart disease is related to an increased risk of dementia has not been comprehensively evaluated. We systematically searched the PubMed, Embase, and Cochrane libraries until 1st November 2017 for longitudinal studies in any language about the relation between coronary heart disease or heart failure and risk of developing dementia. We assessed study quality, performed random effects meta-analysis to obtain pooled effect estimates, and assessed potential publication bias by drawing funnel plots. Among 5,019 unique citations, we identified 16 studies with 1,309,483 individuals regarding coronary heart disease, and 7 studies with 1,958,702 individuals about heart failure. A history of coronary heart disease was associated with 27% increased risk of dementia (pooled relative risk (RR) [95% confidence interval (CI)]: 1.27 [1.07-1.50]), albeit with considerable heterogeneity across studies (I^2=80%). When limiting meta-analysis to prospective population-based cohorts, the pooled estimate was similar (RR 1.26 [1.06-1.49]; 9 studies), and highly consistent across studies (I^2=0%). Heart failure was associated with 60% increased risk of dementia (pooled RR 1.60 [1.19-2.13]), with moderate overall heterogeneity (I^2=59%) again absent among the prospective population-based cohorts (I^2=0%, RR 1.80 [1.41-2.31]; 4 studies). Funnel plot asymmetry appeared explicable by heterogeneity in study design for coronary heart disease, but was also consistent with reporting bias for heart failure. In conclusion, coronary heart disease and heart failure are associated with moderate increases in the risk of dementia. However, results were heterogeneous, and the number of studies about heart failure limited, requiring further longitudinal study with detailed cardiac phenotyping and thorough outcome assessment to delineate these associations.

INTRODUCTION

Coronary heart disease, heart failure, and dementia are among the leading causes of death and disability,[1,2] and often co-occur in the ageing population. The importance of late-life complications of cardiovascular disease has amplified with the advances in cardiovascular medicine over the past decades. Mortality due to coronary heart disease has plunged since its peak in the early 1960s, largely due to improvements in acute treatment and secondary prevention.[3] Similarly, the prognosis with heart failure has improved with better medical treatment and cardiac resynchronization therapy.[3,4] Though great improvements in health care, these developments now render patients with cardiovascular disease susceptible to diseases that have their incidence peak in late-life, such as dementia.

The brain is a highly vascularised organ, receiving 15% of cardiac output and accounting for about 20% of the body's total oxygen consumption despite comprising less than 3% of body weight,[5] and it may therefore be particularly vulnerable to impairment in blood flow. The now well-established importance of cardiovascular risk factors in prevention of dementia, including Alzheimer's disease,[6,7] further suggests that patients with manifest cardiovascular disease may be at increased risk of developing dementia years or even decades later. Because of the urgency for timely intervention to prevent dementia,[8] this could hold important implications for focused preventive strategies.[9] However, evidence from longitudinal studies linking coronary heart disease and heart failure to dementia is fragmented, with inconsistencies between findings, and study populations not seldom too small to detect clinically relevant associations.

We therefore systematically reviewed and meta-analysed the available evidence to determine the association of coronary heart disease and heart failure with future risk of dementia and clinical Alzheimer's disease.

METHODS

Search strategy

We conducted a systematic search of the literature in PubMed, Embase, and the Cochrane library for studies published through 1st November 2017. We searched for prospective studies of in humans published reported the risk of all-cause dementia or Alzheimer's disease in relation to coronary heart disease (CHD) or congestive heart failure (CHF). We limited our search to original articles, excluding scientific abstracts. No restrictions on date or language were applied. The complete search strategy is presented in Box 1. We further

hand-searched bibliographies of relevant publications, and contacted authors of selected publications to complement the data that were available in the published reports.

PUBMED
((dementia[mesh] *OR* dementia[tiab] *OR* alzheimer*[tiab])
AND
("myocardial ischemia"[mesh] *OR* ((myocard*[tiab] *OR* heart[tiab]) AND infarct*[tiab]) *OR* "coronary heart disease"[tiab] *OR* "coronary artery disease"[tiab] *OR* "acute coronary syndrome"[tiab] *OR* "angina pectoris"[tiab] *OR* "myocardial ischemia"[tiab] *OR* "coronary artery obstruction"[tiab] *OR* "coronary artery atherosclerosis"[tiab] *OR* "coronary artery thrombosis"[tiab]
OR "cardiac output, low"[mesh] *OR* cardiomegaly[mesh] *OR* cardiomyopathies[mesh] *OR* "heart failure"[mesh] *OR* "ventricular dysfunction"[mesh] *OR* "heart failure"[tiab] *OR* "cardiac failure"[tiab] *OR* "cardiac function"[tiab] *OR* "heart function"[tiab] *OR* cardiomyopathy[tiab] *OR* cardiomegaly[tiab])
AND ("0001/01/01"[PDAT] : "2017/11/01"[PDAT]))
NOT systematic review[pt] *NOT* review[pt] *NOT* case rep*OR*ts[pt] *NOT* clinical conference[pt] *NOT* congresses[pt] *NOT* editorial[pt] *NOT* Meta-analysis[pt] *NOT* other animals[mh]

EMBASE
('dementia'/exp *OR* dementia:ab,ti *OR* alzheimer*:ab,ti)
AND
('ischemic heart disease'/exp *OR* ((myocard*:ab,ti *OR* heart:ab,ti) AND infarct*:ab,ti) *OR* 'coronary heart disease':ab,ti *OR* 'coronary artery disease':ab,ti *OR* 'acute coronary syndrome':ab,ti *OR* 'angina pectoris':ab,ti *OR* 'myocardial ischemia':ab,ti *OR* 'coronary artery obstruction':ab,ti *OR* 'coronary artery atherosclerosis':ab,ti *OR* 'coronary artery thrombosis':ab,ti
OR 'heart failure'/exp *OR* cardiomyopathy/exp *OR* cardiomegaly/exp *OR* 'heart failure':ab,ti *OR* 'cardiac failure':ab,ti *OR* 'cardiac function':ab,ti *OR* 'heart function':ab,ti *OR* cardiomyopathy:ab,ti *OR* cardiomegaly:ab,ti)
NOT ([systematic review]/lim *OR* [review]/lim *OR* [conference abstract]/lim *OR* [conference paper]/lim *OR* [conference review]/lim *OR* [editorial]/lim *OR* [erratum]/lim *OR* 'nonhuman'/de) *NOT* [01-11-2017]/sd

COCHRANE
(dementia:ti,ab *OR* alzheimer*:ti,ab)
AND
(((myocard*:ti,ab *OR* heart:ti,ab) AND infarct*:ti,ab) *OR* "coronary heart disease":ti,ab *OR* "coronary artery disease":ti,ab *OR* "acute coronary syndrome":ti,ab *OR* "angina pectoris":ti,ab *OR* "myocardial ischemia":ti,ab *OR* "coronary artery obstruction":ti,ab *OR* "coronary artery atherosclerosis":ti,ab *OR* "coronary artery thrombosis":ti,ab
OR "heart failure":ti,ab *OR* "cardiac failure":ti,ab *OR* "cardiac function":ti,ab *OR* "heart function":ti,ab *OR* cardiomyopathy:ti,ab *OR* cardiomegaly:ti,ab)

Box 1. Search terms included for each library search

Study selection

We imported all retrieved records into an EndNote (Clarivate Analytics) library and two investigators independently screened all articles for eligibility, using the following inclusion criteria: 1) cohort studies, or longitudinal studies conducted with routinely collected healthcare data (e.g. national medical registries or insurance databases), 2) determinant CHD (i.e. myocardial infarction with or without angina or coronary revascularisation), or CHF, and 3) report of incident dementia diagnosis as the outcome (i.e. at least all-cause dementia or Alzheimer's disease as its most common subtype). We chose all-cause dementia as the

main outcome measure of interest for this meta-analysis, because a syndrome diagnosis of dementia can be defined with high consistency across studies, and is less dependent on advanced diagnostic testing which is often not feasible in large (population-based) studies. Yet, we acknowledge the importance of various neuropathology underlying the clinical picture of dementia, in particular as heart disease may relate stronger to cerebrovascular pathology than to other neuropathology. To provide more insight in the association of CHD and CHF with dementia independent of manifest cerebrovascular disease, we therefore adopted a clinical diagnosis of Alzheimer's disease (per study protocol) as a secondary outcome measure. If multiple results were reported for the same cohort, we preferred the longer follow-up duration,[10,11] longer follow-up along with more comprehensive assessment of exposure,[12,13] larger number of incident dementia cases,[14-17] most contemporary data,[13] or the study in which selection bias was considered least likely.[18] In case of disagreement between assessors, consensus was reached through discussion.

Data extraction

Study characteristics were extracted from the identified reports independently by two researchers. The extracted information included year of publication, study period, study design, study population, description of the (ascertainment methods for) determinants and outcome, covariates that were adjusted for, follow-up time, number of observed events, and effect estimates with precision estimates (i.e. confidence interval or standard error).

Quality assessment

We critically appraised all selected studies, and formally assessed their quality by using a modification of the Newcastle-Ottawa-Scale,[19] in line with prior recommendations for quality assessment of observational studies.[20] Two independent researchers (FJW and RAS) scored the quality of each study on the following criteria: 1) study design, including source population, and sampling; 2) ascertainment methods for CHD and CHF; 3) incorporation of cognitive screening at baseline; 4) ascertainment methods for dementia; 5) adjustment for potential confounding factors; 6) follow-up duration; 7) attrition. Details of these criteria and rating categories are shown with Table 1. Discrepancies between researchers in quality assessment were solved through consensus meeting.

Analysis

On the basis of expected differences in study populations and methodology, we used inverse variance weighted random effects models to pool the log transformed risk ratios and hazard ratios from primary studies. If multiple models were presented within a study, we selected the multivariable model in each study for meta-analysis. When relative risk (RR) estimates were presented in subgroups only (e.g. by sex), we first meta-analysed the within study

results using fixed effects models. We formally assessed for heterogeneity between studies (Cochran's Q statistic) to determine the share of variation across studies that was due to heterogeneity rather than chance (Higgins' I^2 statistic),[21] and interpreted heterogeneity as probably of minor importance (<40%), moderate (30-60%), substantial (50-90%), or considerable (75-100%), in line with Cochrane recommendations. Publication bias was investigated using funnel plots, and formally tested using Egger's test,[22] in accordance with prior recommendations for interpretation of visual (a)symmetry.[23]

Sensitivity analyses were performed to assess the influence of each individual study, omitting the studies with the largest weight on the overall result one by one (to a minimum of three). We performed additional sensitivity analyses on the basis of study quality criteria, by 1) assessing results from population-based cohort studies only, 2) limiting analyses to studies with adjustment for at least age, sex, and cardiovascular risk factors (Table 1 – adjustment score = 2), 3) limiting analyses to studies with refined outcome assessment (Table 1 – outcome score = 2), and 4) using an alternative case definition of Alzheimer's disease that was reported as sensitivity analysis in one registry study,(24) in an attempt to harmonize case definition of Alzheimer's disease across included studies about heart failure. All analyses were performed using the "meta"-package (version 4.8-4) of the statistical software R, version 3.4.2.

RESULTS

Of 5,019 unique citations that were identified through our search, we included 16 studies reporting the association between CHD and dementia, and 7 reports describing the association between CHF and dementia. The flow diagram illustrating the selection of these studies is presented in Figure 1.

Coronary heart disease

Characteristics of the 16 studies that reported the associations of CHD with future risk of dementia are presented in Table 2. The total number of participants was 1,309,483, with mean age at study entry ranging from 62.1 to 81.5 years, and studies generally including more women than men (overall: 56.6% women). Three of these studies assessed conversion from mild cognitive impairment to dementia,[25-27] while 13 determined risk of dementia in cognitively healthy populations,[11,13,14,18,28-36] predominantly embedded in prospective population-based cohort studies.[11,13,14,18,28,30,31,33,34] Most studies included a history of myocardial infarction as its determinant, generally determined by interview, often with verification in medical records, and sometimes aided by electrocardiography (Table 2).

Study author (acronym, country)	Sampling	Exposure ascertainment	Baseline screening	Adjustment	Outcome assessment	Follow-up duration	Attrition	Total score
Adelborg (Denmark)	1	0	0	2	1	1	2	7
Haugarvoll (Norway)	0	0	1	0	1	0	1	3
Haring (WHIMS, USA)	0	0	1	2	1	1	2	7
Hayden (Cache County, USA)	2	0	1	2	1	0	0	6
Ikram (RS, The Netherlands)	2	1	1	2	2	1	2	11
Jefferson (FHS, USA)	1	1	1	2	2	1	2	10
Kahn (Bronx Ageing Study, USA)	2	1	1	0	1	1	1	7
Kuller (CHS, USA)	1	1	1	2	2	1	0	8
Kuo (Taiwan)	1	0	0	1	1	1	0	4
Li (China)	1	1	1	1	1	0	1	6
Lipnicki (Sydney MAS, Australia)	2	0	1	1	1	0	2	7
Nesteruk (Poland)	0	0	1	0	1	0	0	2
Noale (ILSA, Italy)	1	1	1	2	1	1	1	8
Qiu (Kungsholmen, Sweden 2005)	2	1	1	2	2	0	2	10
Qiu (Kungsholmen, Sweden 2006)	2	1	1	2	2	1	2	11
Rusanen (CAIDE, Finland)	1	1	1	2	2	1	0	8
Satizabal (FHS, USA)	1	1	1	1	2	0	2	8
Solfrizzi (ILSA, Italy)	1	1	1	0	1	0	0	4
Sundbøll (Denmark)	1	0	0	2	1	1	2	7

Table 1. Quality assessment. Scoring criteria were defined as follows: **Sampling:** 2=population-based study, 1=embedded within population-based study (<75% of attendants included), 0=selected cohort or not described; **Exposure ascertainment:** 1=study measurement, or medical records, 0=interview, written self-report, registry, or no description; **Baseline screening for outcome:** 1=yes, 0=no; **Adjustment:** 2=includes cardiovascular risk factors, 1=at least age and sex adjusted, 0=crude estimates or not described; **Outcome assessment:** 2=re-examination and surveillance through medical records and death certificates, 1=independent blind assessment at re-examination or registry data only, 0=self-report or not described; **Follow-up duration**: 1=adequate (≥5 years), 0=short (<5 years); **Attrition:** 2=<15%, 1=15-24%, 0=≥25% or not described; **Overall quality score:** sum of the scores of the individual quality criteria (range 0 to 11). RS=Rotterdam Study; FHS=Framingham Heart Study; CHS=Cardiovascular Health Study; WHIMS=Women's Health Initiative Memory Study; MAS=Memory and Ageing Study; ILSA=Italian Longitudinal Study on Aging; CAIDE=Cardiovascular Risk Factors, Aging and Dementia.

First author	Study cohort	Publication year	Study period	Sample size	Mean age (SD)	Sex (% men)	Ascertainment of determinant	Ascertainment of outcome	Follow-up[§] (years)	Outcome (N)
Coronary heart disease										
Kahn[11]	Bronx Aging Study, USA	1996	1980-NR	459	79	35.3%	Hx of MI (interview & physical examination & ECG)	Re-examination with 1.5 year intervals & hospital records & death certificates & autopsy; NR	Max. 10	NR, ±56
Kuller[14]	CHS, United States	2003	1991-1999	3,608	73	40.9%	Hx of MI (interview & medical records)	Annual re-examination & hospital records	Max. 8	480
Solfrizzi*[27]	ILSA, Italy	2004	1992-1997	139	80.7 (2.5)	49.6%	Hx of MI+AP (interview & medical records & ECG)	Re-examination after 3.5 years; DSM-IIIR; NINCDS-ADRDA	3.5	15
Qiu[28]	Kungsholmen, Sweden	2005	1991-1997	1,301	81.5	25.0%	Hx of IHD (ICD coding from registry)	re-examination & medical records & death certificates; DSM-IIIR; NINCDS-ADRDA	Max. 6	350
Haugervoll[29]	Parkinson Disease, Norway	2005	1992-1997	171	71.2	49.1%	Hx of MI (interview & medical examination, record verification)	Re-examination after 4 years; DSM-IIIR	4	43
Hayden[30]	Cache County, USA	2006	1995-1999	3,264	74.0 (6.4)	41.8%	Hx of MI (interview)	Re-examination after 3 years; DSM-IIIR; NINCDS-ADRDA	3.2	185
Ikram[31]	Rotterdam, the Netherlands	2008	1990-2005	6,347	68.7	41.1%	Hx of MI (interview & medical records & ECG)	4-5 year re-examination and surveillance; DSM-IIIR; NINCDS-ADRDA	9.3	613
Li*[25]	Chinese community sample	2011	2004-2009	837	NR, ±67[†]	41.6%[†]	Hx of MI (interview & medical records & ECG)	Annual re-examination; DSM-IV; NINCDS-ADRDA / NINCDS-AIREN	Max. 5	298
Haring[32]	WHIMS, USA	2013	1996-2008	6,455	65-79	0%	Hx of MI (interview)	Annual re-examination & semiannual questionnaire	8.6	171
Noale[34]	ILSA, Italy	2013	1992-2000	2,501	71.3 (5.3)	43.7%	Hx of MI (interview & ECG)	2-yearly re-assessment	7.8	194
Lipnicki[33]	Sydney MAS, Australia	2013	2005-2008	889	78.6 (4.8)	45.9%	Hx of MI+AP (interview)	Re-examination at 2 years	2.0	23
Rusanen[18]	CAIDE, Finland	2014	1998-NR	738	68	37.6%	Hx of MI (interview & registry ICD-coding)	Re-examination after 8-10 years & registry data (ICD) & medical records; DSM-IV; NINCDS-ADRDA	7.8	46

Kuo[35]	Taiwan registry (non-diabetic)	2015	2000-2011	67,066	62.1 (11.4)	48.4%	Hx of CAD (ICD)	National Health Insurance registry (ICD)	Max. 11	3632
Nesteruk*[26]	Memory clinic, Poland	2015	2010-2014	101	62.7	42.6%	Hx of IHD (interview & medical records)	Re-examination after 6, 12, 24 months; NIA/AA	2	17
Satizabal[13]	Framingham, USA	2016	2004-2008	2,090	72 (9)	44%	Hx of CHD (interview & medical records & ECG)	4-year re-examination and surveillance; DSM-IV; NINCDS-ADRDA	Max. 5	NR
Sundbøll[36]	Danish registry	2017	1980-2012	1,213,517	67	65.9%	ICD-coding	Danish National Patient Registry and Central Psychiatric Registry	7.7-9.8**	85,390
Heart failure										
Haugervoll[29]	Parkinson Disease, Norway	2005	1992-1997	171	71.2	49.1%	Hx of CHF (interview & medical examination, record verification)	Re-examination after 4 years; DSM-IIIR	4	43
Qiu[16]	Kungsholmen, Sweden	2006	1991-2002	1,301	81.5	25.0%	Hx of CHF (interview & medical examination & registry data)	3-yearly re-examination & medical records & death certificates; DSM-IIIR; NINCDS-ADRDA	5.0	440
Haring[32]	WHIMS, USA	2013	1996-2008	6,455	65-79	0%	Hx of CHF(interview)	Annual re-examination & semiannual questionnaire	8.6	171
Noale[34]	ILSA, Italy	2013	1992-2000	2,501	71.3 (5.3)	43.7%	Hx of CHF (interview & medical examination)	2-yearly re-assessment	7.8	194
Rusanen[18]	CAIDE, Finland	2014	1998-NR	738	NR	37.6%	Hx of CHF (interview & registry ICD-coding)	Re-examination after 8-10 years & registry data (ICD) & medical records; DSM-IV; NINCDS-ADRDA	NR	46
Jefferson[12]	Framingham, USA	2015	2002-NR	1,039	69 (6)	47%	Cardiac index (MRI)	4-year re-examination and surveillance; DSM-IV; NINCDS-ADRDA	7.7	32
Adelborg[24]	Danish registry	2016	1980-2012	1,946,497	77 (IQR 69-84)	52%	ICD-coding	Danish National Patient Registry and Central Psychiatric Registry	2-6.5**	148,541

Table 2. Identified studies. *Conversion from mild cognitive impairment to dementia; §Mean or median follow-up duration, unless indicated otherwise; †Reported age and sex in those who remained with mild cognitive impairment or progressed to Alzheimer's disease, excluding other types of dementia or loss to follow-up; **Reported median follow-up duration in the exposed cohort (lower range), and in the unexposed cohort (upper range); NR=not reported; RS=Rotterdam Study; FHS=Framingham Heart Study; CHS=Cardiovascular Health Study; WHIMS=Women's Health Initiative Memory Study; MAS=Memory and Ageing Study; ILSA=Italian Longitudinal Study on Aging; CAIDE=Cardiovascular Risk Factors, Aging and Dementia; MI=myocardial infarction; AP=angina pectoris; CHF=congestive heart failure; IHD=ischaemic heart disease.

Assessment of dementia varied across studies from registry data only, or at re-examination only, to re-examination with complementary surveillance of medical records (Table 2). Mean follow-up time ranged from 2 to 9.3 years, and the number of dementia cases included in the analyses ranged from 15 to 85,390.

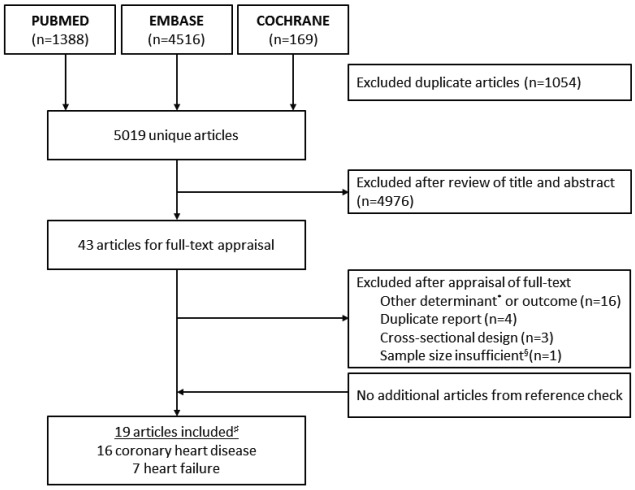


Figure 1. Flow diagram of the literature search. *For one study among Medicare participants in the United States,[48] the authors could report only estimates for a composite measure of heart disease, and one Dutch population-based study excluded participants with clinical heart failure.[37] §In the HYVET trial only 13 (0.4%) participants had heart failure at baseline.[49] ♯Four articles described the associations of both coronary heart disease and heart failure with dementia.

Overall, history of CHD was associated with an increased risk of all-cause dementia (relative risk (RR) [95% confidence interval]: 1.27 [1.08-1.50]; Figure 2), but with considerable heterogeneity across studies (I^2 [95% CI] 80% [66-88%], P<0.0001). Among prospective population-based cohort studies only, heterogeneity was minor, whilst the pooled risk estimate was broadly unchanged (I^2=0%, RR 1.26 [1.08-1.47]). Risk estimates were somewhat higher after exclusion of the highest weighted study,[36] but remained similar with sequential exclusion of the highest weighted studies thereafter (Table 3. Findings were similar for studies that were judged to have made sufficient adjustment for confounding by cardiovascular risk factors, or had in-person outcome assessment in combination with record data (Table 3). Among eight studies that reported estimates for both all-cause dementia and Alzheimer's disease separately, the pooled effect estimate for Alzheimer's disease only was lower (RR 1.07 [0.90-1.28]; Figure 2), albeit less marked in the prospective population-based cohorts (RR 1.23 [1.01-1.50]). The funnel plot of all thirteen studies displayed asymmetry (Figure 4A; *P*-value from Egger test = 0.04), but this was not seen among the nine prospective population-based cohorts (Figure 4C; *P*-value = 0.66).

Three studies reported CHD as a determinant for conversion from mild cognitive impairment to all-cause dementia (2) and Alzheimer's disease (1), respectively. All reported non-significantly increased risks associated with CHD (HR 1.71, 0.32-6.78; RR 1.39, 0.60-3.26; and HR 1.05, 0.67-1.65, respectively).[25-27] Because of the differences in outcome measure in this limited number of studies, we did not meta-analyse these results.

Heart failure

Characteristics of the seven studies investigating CHF and dementia are presented in Table 2.[12,16,18,24,29,32,34] A total number of 1,958,702 participants were included, with mean age at study entry ranging from 69 to 81.5 years, and including 48.2% women. Most studies were embedded in prospective population-based studies.[12,16,18,34] Continuous measures of cardiac function and/or diagnosis of CHF were obtained using interview and ICD-coding, or on one occasion cardiac MRI[12] (Table 2). In two studies using ICD-coding, diagnosis was verified by medical examinations.[16,29] Assessment of dementia varied across studies from registry data only, or re-examination only, to re-examination with complementary use of medical records (Table 2). Mean follow-up time ranged from 4 to 8.6 years, and during this period 32 to 148,541 participants had a diagnosis of dementia. Of meta-analysed studies, five reported estimates for all-cause dementia and Alzheimer's disease. Diagnosis of Alzheimer's disease was based on the NINCDS-ADRDA criteria in most studies, except one registry.[24]

Among seven studies that were included in the meta-analysis, history of CHF was associated with an increased risk of all-cause dementia (RR 1.59 [1.19-2.13]; Figure 3). There was moderate heterogeneity across studies (I^2 [95% CI] 58% [5-82%]), which was not present among the prospective population-based studies (I^2 = 0% [0-69%]; pooled RR 1.80 [1.41-2.31]). Results were grossly unaltered when sequentially excluding the studies with the largest weight, or when limiting meta-analysis to studies with rigorous confounding, or with in-person outcome assessment in combination with record data (Table 3).

Among the 5 studies that reported estimates for both all-cause dementia and Alzheimer's disease separately, the pooled effect estimate for Alzheimer's disease only was slightly lower (RR 1.44 [0.95-2.16]; Figure 3), albeit with substantial heterogeneity across studies (I^2 = 74% [35-90%]). Heterogeneity was somewhat reduced when applying a case definition of Alzheimer's disease to the report of Adelborg et al. that was more in line with other studies, as also suggested by the authors in their original report (I^2 55% [0-84%]).[24] The pooled effect estimate thereby changed to 1.46 (95% CI 1.07-1.99). The funnel plot suggested studies with smaller effect estimates for CHF could have been underreported (Figure 4B; *P*-value from Egger test = 0.03), although again, asymmetry seemed less profound among the (limited number of) purposefully designed prospective population-based studies (Figure 4D).

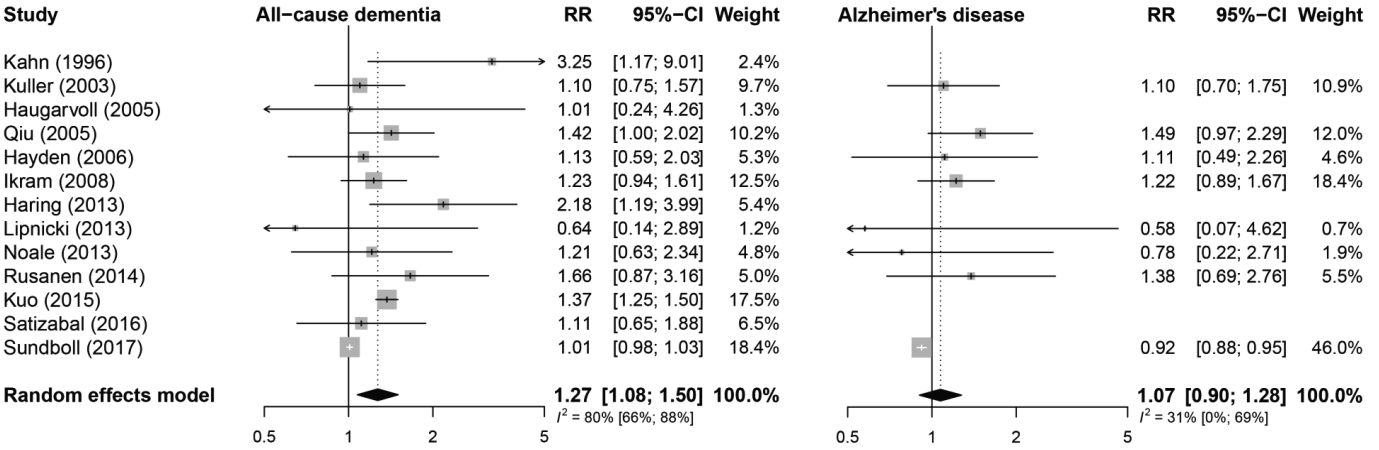


Figure 2. Coronary heart disease and dementia. Forest plots show per study and pooled associations of coronary heart disease with all-cause dementia (left) and Alzheimer's disease (right).

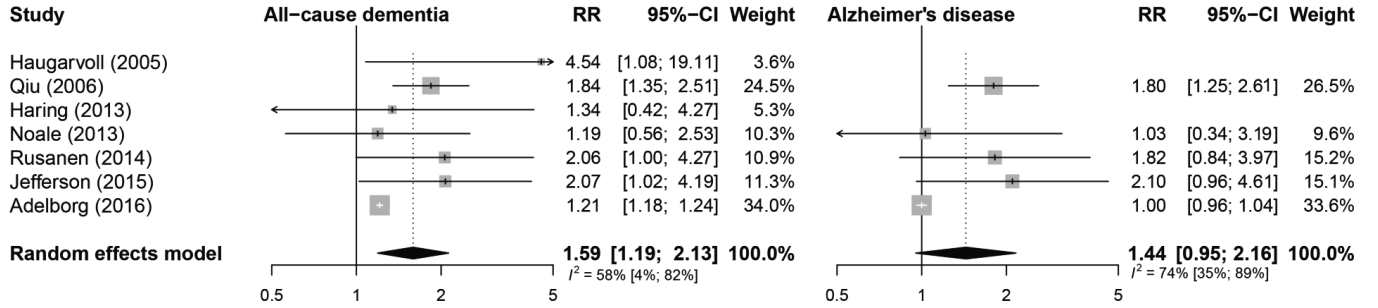


Figure 3. Heart failure and dementia. Forest plots show per study and pooled associations of heart failure with all-cause dementia (left) and Alzheimer's disease (right).

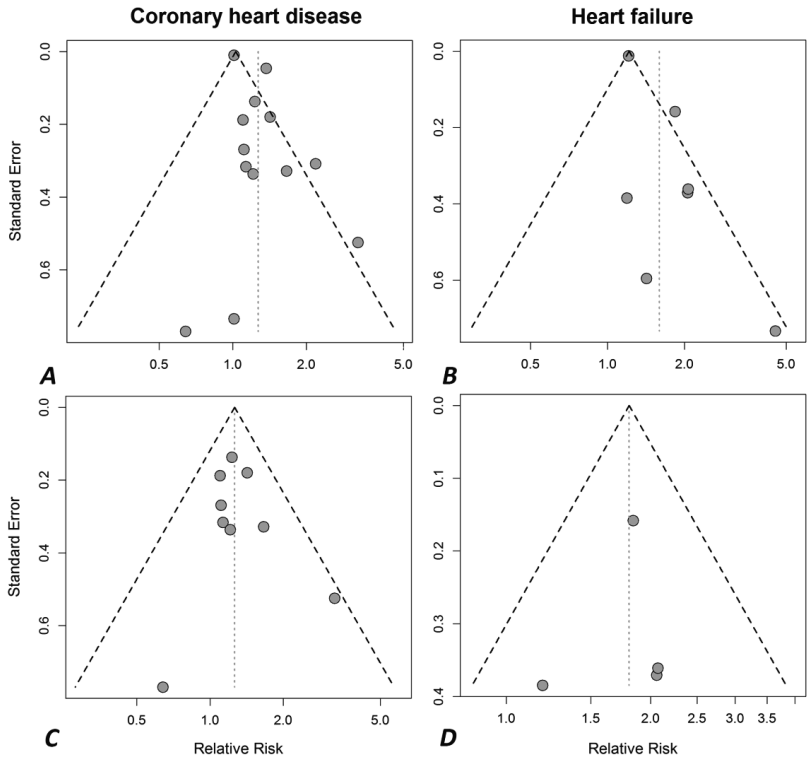


Figure 4. Funnel plots for studies about coronary heart disease (A and C) and heart failure (B and D). The top row includes all studies (A and B); the bottom row shows only prospective population-based cohorts (C and D).

	Coronary heart disease Relative risk (95% CI)	**Heart failure** Relative risk (95% CI)
Overall pooled estimate	1.27 (1.07-1.50)	1.60 (1.19-2.13)
Excluding studies by weight*		
-1 study	1.35 (1.25-1.46)	1.83 (1.44-2.33)
-2 studies	1.30 (1.12-1.51)	1.82 (1.24-2.67)
-3 studies	1.33 (1.12-1.60)	1.73 (1.10-2.72)
-4 studies	1.32 (1.05-1.65)	1.64 (0.81-3.30)
-5 studies	1.42 (1.09-1.85)	n/a
-6 studies	1.52 (1.12-2.07)	n/a
-7 studies	1.37 (0.99-1.91)	n/a
-8 studies	1.48 (0.99-2.22)	n/a
-9 studies	1.39 (0.76-2.55)	n/a
-10 studies	1.45 (0.52-4.02)	n/a
Quality criteria‡		
Confounding adjustment (score =2)	1.22 (1.03-1.45)	1.52 (1.16-1.99)
Outcome assessment (score =2)	1.25 (1.06-1.48)	1.90 (1.46-2.47)
Follow-up duration (score = 1)	1.30 (1.07-1.59)	1.52 (1.16-1.99)

Table 3. Sensitivity analyses on the basis study weight and quality according to specified criteria. *sequential exclusion of the studies with the highest weight from the analyses; for the assigned weights per study please see Figure 2 and Figure 3; ‡for the quality scores by individual study please see Table 1. CI=confidence interval.

DISCUSSION

In this comprehensive systematic review and meta-analysis of longitudinal studies, we compiled the current evidence on the association between CHD and future risk of dementia in 1,309,483 individuals from 16 cohort studies, and the association between CHF and future risk of dementia in 1,958,702 individuals from 7 cohorts. On the basis of available evidence, CHD and CHF are associated with mild to moderate increases in the risk of dementia.

Despite these overall associations, there was considerable heterogeneity among included studies. This may in part be explained by study design, as consistent associations emerged from purposefully designed longitudinal studies, which could rely on in-person examinations for assessment of heart disease (e.g. using imaging data or ECG) and dementia. Much of the heterogeneity was, in fact, attributable to the results from one Danish registry.[24,36] Misclassification of exposure in registry studies may dilute the observed risk estimates, as could inaccuracy of the outcome assessment (in particular in the presence of false-positive diagnoses). Of note, the positive predictive values of a diagnosis of all-cause dementia and Alzheimer's disease in this particular registry are 86% and 81%, respectively, whereas diagnostic sensitivity of registry data is often unknown.[36] Although this heterogeneity could contribute to asymmetry of the funnel plots (and in fact more homogeneous study results went along with more symmetrical plots for CHD), we caution that the observed asymmetry, in particular for heart failure, could also reflect reporting bias. Funnel plots were design for assessment of randomised controlled trials, rather than observational studies, and their interpretation in the presence of methodological inconsistency and potential biases is challenging.[23] This supports refining analysis on the basis of methodological rigour of original studies, or suspicion of true (biological) heterogeneity. In any case, the limited number of heart failure studies calls for additional evidence to further delineate its association with risk of dementia.

For CHD, the assessment strategy differed between studies, most notably in the use of electrocardiography to confirm a diagnosis of MI. Objective confirmation of a diagnosis reduces information bias, could allow identification of asymptomatic MI,[31] and provide information about infarct localisation. For CHF, only one study quantified cardiac function, using MRI, with the additional benefit of assessing cardiac dysfunction in relation to dementia on a continuous scale.[12] A similar quantitative approach in the Rotterdam Study showed that diastolic dysfunction, as measured by echocardiography, was associated with an increased risk of dementia among 3,291 individuals without clinical CHF.[37] As this report included asymptomatic individuals only, it was not included in the current meta-analysis. Nevertheless, these studies jointly illustrate the benefit of quantifying cardiac function in

relation to (markers and symptoms of cerebrovascular disease and) neurodegeneration, and suggest that potential detrimental effects of cardiac dysfunction on brain health are not limited to symptomatic CHF.

Part of the observed heterogeneity between studies may have arisen from differences in outcome assessment and follow-up strategy. As eluded to above, apart from registry-based diagnosis,[24,26] all studies incorporated interval assessments for diagnosis which ranged from every 6 months to every 4 years. As individuals with (mild) cognitive impairment are less likely to attend study follow-up visits, shorter intervals to re-examination and supplementary surveillance strategies can contribute to higher diagnostic sensitivity in some studies compared to others. When examining Alzheimer's disease as the most common subtype of dementia in meta-analysis, we faced substantial differences in means of subtype diagnosis between studies. For example, in the large Danish registry-based study, 34.6% of dementia cases were attributed to Alzheimer's disease,[24] compared to 61-81% in the population-based cohorts. The association between CHF and Alzheimer's disease became stronger (adjusted HR 1.16 [1.14–1.20]), when the authors of the Danish study reclassified the large share of ICD-coded unspecified dementia as Alzheimer's disease in a sensitivity analysis (resulting in 77.7% of dementia being classified as Alzheimer's disease). This highlights the importance of uniform criteria for case definition, as well as a lack of diagnostic certainty about dementia subtyping with various accumulating pathologies and the multifactorial aetiology of dementia and what is called (late-onset) Alzheimer's disease. Notwithstanding various underlying neuropathology, a syndrome diagnosis of dementia provides a more consistent primary outcome measure in population studies, which is likely preferable for comparison over heterogeneous clinical disease subtypes. Most studies used the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) criteria for all-cause dementia, although this wasn't specified in some,[11,14,32-34] or unverifiable given the registry-based nature of others.[24,35-36]

Disease of the heart and brain are both common in the elderly population. This meta-analysis suggests that this is no coincidental co-occurrence, but that heart and brain are in fact linked in such a way that being diagnosed with CHD or CHF predisposes to development of dementia. This might aid in identifying people prone to cognitive decline, and from an aetiological perspective emphasises the need to unravel the mechanisms underlying the link between heart disease and cognition, which may become all the more evident with improving life expectancy of patients with heart disease. Potential explanations include cerebral hypoperfusion and hypoxia (either due to cardiac arrhythmias or haemodynamic consequences of impaired cardiac function),[38] cerebral ischaemia (e.g. thromboembolic complications),[36,39] shared aetiology, effects of a pro-inflammatory state,[40] direct effects of

natriuretic peptides,[41] or related to (vascular) amyloid.[42,43] Some of these could be specific to either CHD or CHF, while others may overlap. For instance, a substantial share of patients with CHD experiences decline in cardiac function as a consequence of ischaemic heart disease. CHF may thus be a mediator in the association between CHD and dementia, supported by higher risk estimates for CHF in general, as well as in individuals with (repeated admissions for) heart failure following myocardial infarction in one of the included studies.[36] Yet, other studies included in this meta-analysis investigated either determinant separately, precluding any firm conclusion about their contribution relative to one another.

The vast majority of identified studies in this systematic review were community-based, and none of the study populations were recruited from cardiology clinics or coronary care units. This is in line with earlier observation that study of cognition in patients presenting to clinics with heart disease is scarce.[9] Given the emerging link between heart disease and cognition, a multi-diagnostic approach that involves cardiologists, neurologists, and geriatricians may benefit risk stratification and medical decision-making, leading to tailored intervention of patients at particular high risk of cognitive decline or dementia.[9,44] Increased attention for cognitive deficits in this at-risk population could aid in identifying potential differential effects of acute treatment strategies on cognition,[45] and development of targeted, more effective preventive strategies. Such an approach would facilitate investigation of dementia risk by type (e.g. type 1 versus type 2 myocardial infarction, or CHF with preserved versus reduced ejection fraction),[46] or severity of heart disease (e.g. by imaging or serum markers) to further unravel the biological underpinning of the presented associations.

Strengths of our study include the comprehensive literature search, without any restriction in date or language of published studies. In addition, we formally assessed quality of studies, integrating recommendations for evaluating potential bias in cohort studies,[20] and dementia research.[40] Some limitations also need to be taken into account. First, none of the included studies enrolled participants instantly at time of CHD or CHF diagnosis, potentially causing selection bias. As more severely impaired patients would have been less likely to enrol at a later stage, this most likely resulted in underestimation of a causal association with dementia. An integrative approach of cognitive work-up along with secondary prevention from the moment of diagnosis may alleviate this limitation in future studies. Second, despite best efforts, we could not obtain risk estimates for Alzheimer's disease from all studies, potentially leading to selection bias. Third, in the presence of substantial heterogeneity, random effects models can give disproportionate weight to smaller studies, rendering them not necessarily more conservative than fixed effects models. We believe that the anticipated (and observed) heterogeneity between studies in our systematic review merits the use of the former, but stress that with additional evidence emerging, periodic updates of this

report will be needed to refine risk estimates. At present, the number of included studies remains relatively limited, hampering interpretation of funnel plots, both visually and by means of formal statistical test,[23] as well as identification of sources of heterogeneity through for example meta-regression. Fourth, associations between cardiac disease and risk of dementia may have changed over time with improved acute care and secondary prevention, emphasising the continuous need for contemporary studies. Fifth, available evidence originates from a limited geographical range, warranting future studies that include individuals of non-European descent, and are embedded in healthcare systems beyond the United States or Europe.

In conclusion, on the basis of currently available evidence from longitudinal studies, CHD and CHF are associated with a mild to moderately increased risk of developing dementia. However, substantial heterogeneity among studies and caution for reporting bias among heart failure studies emphasise the need for additional high-quality evidence to establish these associations, identify their potential biological underpinning, unravel characteristics of CHD and cardiac function that could impose higher risk of dementia, and eventually determine the effect of targeted preventive interventions.

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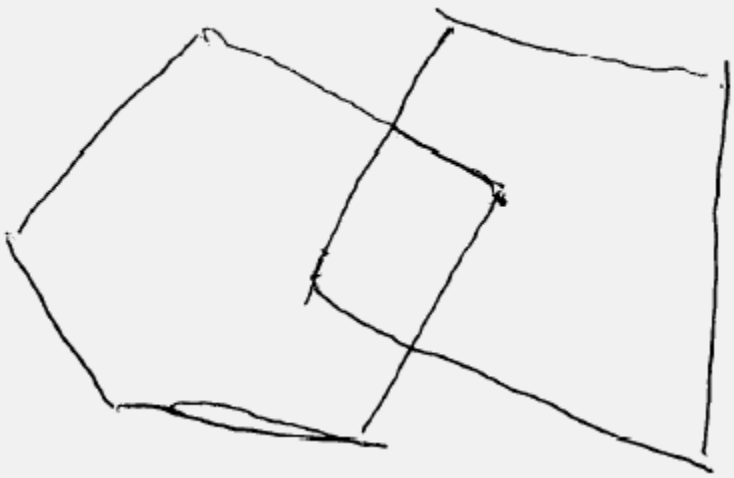
Chapter 4.2

Aortic valve calcification



ABSTRACT

Aortic valve calcification (AVC) is a strong risk factor for cardiac disease and mortality. Its further association with covert brain infarcts suggests that AVC can have direct thromboembolic and hemodynamic consequences on the brain, which may also contribute to dementia. However, no published studies have investigated the relation between AVC and cognitive decline or dementia. We used computed tomography (CT) to quantify AVC in 2428 non-demented participants of the population-based Rotterdam Study (mean age 70 years, 52% women) who underwent CT between 2003 and 2006. Participants were followed for incident dementia until 2015, including detailed cognitive assessment at time of CT and after on average 6 years. We assessed correlation of AVC with calcification in other vessel beds, and determined cognitive decline and risk of dementia with AVC, using linear regression and Cox proportional hazard models. AVC-volume was moderately correlated with volumes of arterial calcification in the coronary arteries, the aortic arch, and the carotid arteries (Spearman's correlation coefficients ranging from 0.29 to 0.32, $P<0.01$). During a median follow-up of 9.3 years, 160 participants developed dementia. We found no association between AVC and risk of all-cause dementia (hazard ratio [95% confidence interval]: 0.89 [0.63-1.26]). Presence of AVC was not associated with change in cognition on repeated cognitive assessment. We observed insufficient dementia cases to determine associations with pure vascular dementia. In conclusion, we found no evidence of an association of AVC with cognitive decline and risk of dementia during prolonged follow-up in the general population.

INTRODUCTION

Aortic valve calcification (AVC) is a strong risk factor for cardiac disease and mortality.[1,2] In addition, AVC has recently been associated with covert brain infarcts,[3] which may indicate direct thromboembolic and hemodynamic consequences on the brain. Thromboembolism and haemodynamic impairment of brain perfusion are implicated in the pathophysiology of dementia, including Alzheimer's disease,[4,5] but no published studies have investigated whether AVC relates to cognition and dementia. We therefore investigated the association between AVC and risk of cognitive decline and dementia in a population-based setting.

METHODS

Study population

This study was embedded in the ongoing Rotterdam Study,[6] a prospective population-based cohort study in middle-aged and elderly persons. Participants are invited for interview and examinations at a dedicated research centre every 4 years. Between 2003 and 2006, all participants were invited to undergo computed tomography for the visualization of vascular calcifications in major arteries, including the aortic root where the aortic valves are located. In total 2,524 participants participated,[7] of whom 44 (1.7%) were excluded because of prevalent dementia or insufficient cognitive screening at baseline. We were unable to measure AVC in 52 (2.1%) participants due to aortic valve replacement, image artifact due to a pacemaker or coronary stent implantation, or bad image acquisition, thus leaving 2,428 (96.2%) participants for analysis in the present study.

Assessment of AVC and arterial calcification

We acquired non-contrast CT-examinations with a multidetector computed tomography (MDCT) scanner (Somatom Sensation 16/64, Siemens, Forchheim, Germany).[7] Using an ECG-gated cardiac imaging protocol, we visualized the aortic root including the aortic valve. We quantified AVC (mm^3), located on the aortic valve leaflets, the base of the cusps, and the annulus,[3,8] using dedicated commercially available software (Syngo.ViaCalciumScoring, Siemens, Germany). The same software was used for quantification of calcification in the coronary arteries, aortic arch, and extracranial carotid arteries. Intracranial carotid artery calcification was quantified using custom-made software, as described previously.[9]

Assessment of cognition

Participants underwent extensive cognitive assessment at time of CT and at one subsequent follow-up visit after on average 6 years. These cognitive assessments included a verbal

fluency task, a letter-digit substitution task, a word-learning test, the Stroop task, and the Purdue pegboard test.[10] For each test, Z-scores were computed for each participant by dividing the difference between the individual cognitive test score and the population mean by the population standard deviation. We also derived a compound measure of global cognition by factor analysis, including all aforementioned tests.[10]

Dementia screening and surveillance

Participants were screened for dementia at baseline and subsequent centre visits using the Mini-Mental State Examination (MMSE) and the Geriatric Mental State Schedule (GMS) organic level.[11] Those with MMSE<26 or GMS>0 underwent further investigation and informant interview including the Cambridge Examination for Mental Disorders of the Elderly. Additionally, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study centre with medical records from general practitioners and the regional institute for outpatient mental healthcare. Available clinical neuroimaging data were reviewed when required for diagnosis of dementia subtype. A consensus panel headed by a consultant neurologist established the final diagnosis according to standard criteria for dementia (DSM-III-R), and Alzheimer's disease (NINCDS-ADRDA). Follow-up until January 2015 was near complete (96.5% of potential person-years).

Other measurements

Information on current smoking habits, and use of blood pressure-lowering or lipid-lowering medication was obtained by interview. Body mass index (BMI) was calculated from measurements of height and weight (weight(kg)/height^2(m)). Systolic and diastolic blood pressure was assessed at the right arm and the mean of two measurements was used in the analyses. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and glucose were measured from fasting blood samples. Diabetes was defined as fasting serum glucose levels ≥7.0 mmol/L or the use of anti-diabetic therapy. We determined history of coronary heart disease (i.e. previous myocardial infarction or revascularization procedure) and heart failure at baseline interview, with verification from medical records.

Analysis

Given the right-skewed distribution of AVC-volume, a natural-log transformation was performed after we added 1.0 mm^3 to the original volumes in order to deal with participants with a naught calcium score [LN(AVC+1.0mm^3)]. Missing data on covariates (maximum 5.7%) were handled using 5-fold multiple imputation. First, we assessed the correlation between AVC and arterial calcification in the coronary arteries, aortic arch, and carotid arteries. We then determined the association between presence of AVC and dementia, using Cox proportional hazards models adjusting for age and sex, and additionally in a second model

for various cardiovascular risk factors (body-mass index, systolic and diastolic blood pressure, use of blood-pressure lowering medication, diabetes, total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medication, smoking, history of coronary heart disease, history of heart failure, and *APOE*-ε4 genotype). In this model, participants were censored within the follow-up period at date of dementia diagnosis, date of death, date of loss to follow-up, or 1st January 2015, whichever came first. We verified that the proportional hazard assumption was met. Second, we calculated tertiles of AVC burden in those with AVC, and determined risk for each tertile compared to the absence of AVC. Finally, we determined change in cognitive test performance in relation to AVC, using linear regression, with adjustments for confounders as described above.

Analyses were performed with IBM SPSS Statistics version 23 (IBM Corporation, Armonk, New York). Alpha level (type 1 error) was set at 0.05.

Characteristics	Study population
Age, years	69.5 (±6.7)
Female	1256 (51.7%)
Body mass index, kg/m^2	27.7 (±3.9)
Systolic blood pressure, mmHg	147 (±20)
Diastolic blood pressure, mmHg	80 (±11)
Serum total cholesterol, mmol/L	5.7 (±1.0)
Serum HDL cholesterol, mmol/L	1.4 (±0.4)
Diabetes	305 (13.3%)
Current smoking	374 (15.9%)
Use of blood-pressure lowering agents	978 (40.9%)
Use of lipid-lowering medication	594 (24.8%)
History of coronary heart disease	197 (8.2%)
History of heart failure	66 (2.7%)
APOE ε4 carrier	620 (26.9%)

Table 1. Population characteristics of the 2,428 participants. Values are means (±standard deviation) for continuous variables or absolute values (%) for categorical variables. HDL=high-density lipoprotein.

RESULTS

Table 1 shows the baseline characteristics of the study population. The mean age at time of CT was 69.5 years, and 51.7% of participants were women. Overall, the prevalence of AVC was 32.9%, but this strongly increased with age from 23.0% at age 60-69 to 70.6% in those ≥90 years. Volume of AVC was correlated with volumes of arterial calcification in the coronary arteries, the aortic arch, and the carotid arteries (Spearman's correlation coefficients ranging from 0.29 to 0.32, P<0.01).

During a median follow-up of 9.3 years (IQR 7.9-9.8), 160 participants were diagnosed with dementia, of whom 126 had Alzheimer's disease. Presence of AVC was not associated with the risk of dementia, either (Table 2). Results were virtually similar for Alzheimer's disease.

Of 2,418 participants who had extensive cognitive assessment at baseline, 1,816 (85.0% of surviving, non-demented participants) had repeated cognitive assessment at follow-up (mean interval 6.0 years, SD 0.5). Presence of AVC was not associated with change in cognitive test performance on any of the performed tests, or with a measure of global cognition (Table 3). This was again similar in analyses per tertile of increasing burden of AVC.

	All-cause dementia N_{dem}/N_{total}=160/2428		**Alzheimer's Disease** N_{dem}/N_{total}=126/2428	
	Model I HR (95%CI)	Model II HR (95%CI)	Model I HR (95%CI)	Model II HR (95%CI)
Aortic valve calcification (presence vs. absence)	0.89 (0.64-1.25)	0.89 (0.63-1.26)	0.88 (0.60-1.29)	0.85 (0.58-1.27)
Per tertile of calcification*				
T1	0.90 (0.55-1.49)	0.91 (0.54-1.52)	1.00 (0.59-1.71)	0.98 (0.56-1.72)
T2	0.89 (0.55-1.44)	0.87 (0.53-1.44)	0.89 (0.52-1.53)	0.83 (0.48-1.46)
T3	0.88 (0.55-1.41)	0.90 (0.56-1.46)	0.77 (0.45-1.32)	0.76 (0.43-1.34)

Table 2. Aortic valve calcification and risk of dementia. *Tertiles of AVC compared to persons without AVC. Model I is adjusted for age and sex, whereas model II is adjusted for age, sex, body-mass index, systolic and diastolic blood pressure, use of blood-pressure lowering medication, diabetes, total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medication, smoking, history of coronary heart disease, history of heart failure, and *APOE*-ε4 genotype. HR=hazard ratio; CI=confidence interval;

	Presence of aortic valve calcification	
	Model I β (95% CI)	Model II β (95% CI)
Cognitive test		
Letter-digit substitution task	-0.011 (-0.099;0.076)	0.006 (-0.085;0.097)
Verbal fluency	0.011 (-0.089;0.111)	0.027 (-0.077;0.131)
Word-learning test	0.0004 (-0.110;0.111)	0.016 (-0.099;0.131)
Stroop	-0.019 (-0.107;0.070)	-0.022 (-0.114;0.070)
Purdue pegboard	-0.017 (-0.169;0.135)	0.004 (-0.155;0.163)
G-factor	0.028 (-0.059;0.114)	0.038 (-0.051;0.127)

Table 3. Aortic valve calcification and change in cognition. Change in standardized cognitive test scores for presence vs. absence of aortic valve calcification. Higher scores indicate better performance for all tests (i.e. Stroop scores are inverted). Model I is adjusted for age and sex, whereas model II is adjusted for age, sex, body-mass index, systolic and diastolic blood pressure, use of blood-pressure lowering medication, diabetes, total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medication, smoking, history of coronary heart disease, history of heart failure, *APOE*-ε4 genotype, and the intervals between CT and cognitive assessments.

DISCUSSION

In this large population-based study we found no association of CT-quantified AVC with cognitive decline or risk of dementia during 10 years of follow-up. Although we are not aware of any other studies investigating AVC in relation to dementia, our findings are thought-provoking in view of the abundance of evidence linking vascular risk factors and atherosclerosis to dementia and Alzheimer's disease .[12] We found lower correlations of AVC with aortic, coronary and cerebral artery calcification, compared to previously described correlations of calcification among these other vessel beds.[13] As arterial calcification in these vessel beds has been associated with dementia previously,[13] our findings suggest that AVC might be a more localized process with, in part, different underlying pathophysiology. Shared risk factors between atherosclerosis and dementia might also contribute less to the development of AVC. In addition, direct thromboembolic complications of AVC may be too limited in duration or severity to result in significant neuronal injury. Nevertheless, with most severe calcification (i.e. stenosis) the brain may still suffer from hemodynamic impairment. Overall, our study sample was relatively healthy and the number of people with stenosis in our sample limited, and associations with valve stenosis may therefore be further explored in future observational studies. Although we were sufficiently powered to detect a moderate effect size of 1.5 with the overall sample for all-cause dementia (α=0.05, β=0.80), we observed insufficient cases of vascular dementia to assess their presumably stronger association with AVC.

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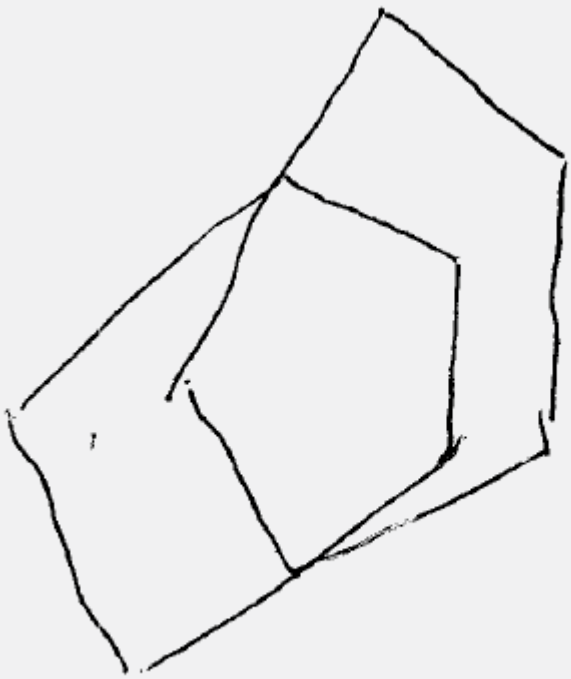
Chapter 4.3

Amyloid in cardiovascular disease



ABSTRACT

Amyloid-β is a hallmark of Alzheimer's disease, and acts in conjunction with cerebrovascular pathology on the risk of dementia. Although amyloid-β is also abundant in the circulation, its systemic effects on various manifestations of vascular disease remain largely unchartered. Between 1990 and 1993 we measured plasma levels of amyloid-β40 and -β42 (Aβ40 and Aβ42) in a random subset of 1,815 participants of the population based Rotterdam Study (mean age 69 years, 62% female). We determined the associations of plasma amyloid with prevalence and 20-year risk of coronary heart disease (CHD), cerebrovascular disease (CeVD), and cause-specific mortality, in comparison to previously established dementia risks. Subclinical atherosclerotic burden was quantified at baseline by carotid ultrasound. At baseline, 78 individuals had dementia, 131 CHD, and 95 CeVD. Higher Aβ40 was associated with the presence of CHD (odds ratio [95% confidence interval] per standard deviation: 1.45 [1.11-1.91]) and CeVD (OR 1.60 [1.17-2.19]), independent of dementia. During follow-up, 304 individuals developed dementia, 141 had CHD, 357 CeVD, and 1,085 died of whom 376 (34.7%) due to cardiovascular causes. Higher Aβ40, but not Aβ42, was associated with risk of death (hazard ratio 1.22 [1.11-1.35]), in particular from cardiovascular causes (HR 1.32 [1.11-1.56]). Individuals with higher baseline Aβ40 were at increased risk of dementia (throughout follow-up), and CHD (up till 10 years), but not CeVD. Lower Aβ42 was strongly associated with prevalent and incident dementia, but not with CHD or CeVD. Neither Aβ40 nor Aβ42 were associated with the burden of subclinical carotid artery atherosclerosis. In conclusion, plasma levels of amyloid-β40, but not -β42, are associated with risk of cardiovascular disease and mortality. These findings imply distinct pathophysiological roles of various length amyloid-β peptides in vascular disease and dementia, and suggest important effects of amyloid-β40 beyond the central nervous system.

INTRODUCTION

Cerebrovascular disease is a major contributor to dementia, including Alzheimer's disease,[1,2] but the precise mechanisms by which vascular disease leads to cognitive decline remain elusive. In particular, few published studies have investigated vascular disease in relation to traditional hallmarks of Alzheimer's disease pathology, notably amyloid-β monomers. Amyloid-β proteins are derived through cleavage of the amyloid precursor protein by β- and γ-secretases. Of the resulting various length hydrophobic amyloid-β monomers, amyloid-β40 (Aβ40) is most abundant in the circulation, whereas amyloid-β42 (Aβ42) is more common in cerebral depositions in patients with Alzheimer's disease. Aβ40 is markedly present in the vascular wall and in platelets, thought to be the primary source of amyloid-β in blood,[3] and form the primary constituent of vascular amyloid deposits in cerebral amyloid angiopathy.

Notwithstanding the wide availability of amyloid in the systemic circulation, prior studies have generally focussed on its presence in the central nervous system. Plasma levels do not necessarily reflect those in the central nervous system,[4] but plasma levels of Aβ42 and to a lesser extent Aβ40 nevertheless relate to dementia risk in the general population.[5-7] Recent reports have linked plasma Aβ40, but not Aβ42, to the presence of coronary artery disease,[4,8] and increased cardiovascular mortality in patients with coronary heart disease.[9] Moreover, one community-based study has reported increased all-cause mortality with higher levels of Aβ40,[10] indicating that (plasma) Aβ40 may be of relevance to pathology in the brain, as well as in other parts of the body. However, via which mechanisms either occurs, and whether cardiovascular and neurodegenerative disease manifest independently is unknown. This calls for further study with follow-up for cardiovascular disease and cause-specific mortality , in addition to dementia, to unravel systemic effects of amyloid-β from those within the central nervous system.

We therefore aimed to determine the association of circulating plasma Aβ40 and Aβ42 with cardiovascular disease (coronary heart disease and cerebrovascular disease) and cause-specific mortality, comparative to their association with dementia, in a population-based setting.

METHODS

Study population

This study is embedded within the Rotterdam Study, a large ongoing population-based cohort study in the Netherlands, with an initial study population of 7,983 participants (78% of invitees) aged ≥55 years from the Ommoord area, a suburb of Rotterdam. The Rotterdam Study methods have been described in detail previously.[11] In brief, participants were interviewed at home and subsequently examined at the research centre for baseline assessment from March 1990 to July 1993. Blood samples were drawn by venepuncture during baseline assessment. Of 7,983 participants, 7,157 (89.7%) visited the research centre for physical examination. Of these, for reasons of efficiency, a random sample of 1,834 individuals was selected for measurement of plasma amyloid-β.

Plasma amyloid measurement

Detailed methods for the amyloid measurements have been described previously.[5] Sodium citrate containing vacutainers were put on ice immediately and centrifuged within 60 minutes after sampling. Aliquots of plasma were stored at −80°C. Plasma concentrations of amyloid-β were determined by a double-antibody 96-well sandwich enzyme-linked immunosorbent assay (ELISA) method (Pfizer, USA). Low fluorescence DELFIA microtitre plates were coated with 100µL of 5µg/mL of 6E10 monoclonal antibody (Signet 9320-10, USA) in carbonate buffer (pH 9·9; Pierce 28 382), for 1 hour at room temperature, and then overnight at 4°C. Plates were then allowed to come to room temperature, and washed with DELFIA wash buffer (Perkin-Elmer, USA). Plates were then blocked with 2% bovine serum albumin and 0.2% Tween-20 in phosphate buffer solution for 1 hour. After the blocking step, plates were washed and then 125µL (amyloid-β42) or 100µL (amyloid-β40) of quality control, standards, or samples were added. Protease inhibitors were added to samples before analysis (Roche 1836145). Plates were incubated overnight at 4°C. After overnight incubation, plates were allowed to come to room temperature, and then washed, after which 100µL of R226 (Aβ1–42; 1:400) or R209 (Aβ1–40; 1:1000) were added and plates incubated at room temperature for 90 minutes. Antibodies were provided by Dr Pankaj Mehta. Plates were then washed with DELFIA buffer and 100µL of Europium labelled streptavidin was added as per manufacturer's instructions (Perkin-Elmer). Plates were incubated in the dark for 1 hour, washed, and incubated for 1 hour with 1:1000 dilution of Europium streptavidin. Plates were then washed and incubated with DELFIA enhancer solution for 2.5 min. Plates were read by time resolved fluorometry from a Wallac model 1420 multi-label counter (Victor2) instrument. Aliquots of pooled patient samples with varying ranges of amyloid-β (low, medium, and high) were frozen and used as quality control samples. Amyloid-β peptide standards were obtained from California Peptide and stored in

dimethyl sulphoxide at −80°C. The mean coefficients of within and between assays variations were 4.4% and 10.1% for amyloid-β40, and 4.9% and 14.8% for amyloid-β42. The detection limits were 10–1000pg/mL for amyloid-β40 and 5–100pg/mL for amyloid-β42.

Screening and surveillance for disease outcomes

Participants underwent extensive examinations at a dedicated research centre at baseline and every four years henceforth, including structured interview about their past medical history, current medication use, 12-lead resting electrocardiogram (ECG), and cognitive screening using the Mini-Mental State Examination and the Geriatric Mental State Schedule organic level.[11] Those with a Mini-Mental State Examination score <26 or Geriatric Mental State Schedule >0 subsequently underwent examination and informant interview using the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX).[12] The information from in-person screening was supplemented by data from the electronic linkage of the study database with medical records from all general practitioners, nursing homes, and the regional institute for outpatient mental health care. With this linkage, the entire cohort is continuously monitored for verification of diagnosis at centre visits, and detection of interval cases of disease between centre visits. For all suspected cases of dementia, a consensus panel led by a consultant neurologist, decided on the final diagnosis in accordance with standard criteria for dementia (DSM-III-R) and Alzheimer's disease (NINCDS-ADRDA).[12]

Coronary heart disease included myocardial infarction and coronary revascularisation procedures. Myocardial infarction was defined as pathology findings of an acute myocardial infarction within 28 days of death, or a rise/fall in cardiac biomarkers, and/or objective indicative ECG changes, and preferably the presence of symptoms or signs (e.g. cardiac pain, cardiogenic shock).[13] Prevalence of myocardial infarction at baseline was based on verification in medical records of either self-reported myocardial infarction or ECG abnormalities indicative of prior myocardial infarction. Diagnosis of myocardial infarction during follow-up is based on information from medical records.[13] A history of coronary artery bypass grafting or percutaneous coronary interventions for coronary heart disease was based on self-reported history with verification in the medical records.[13] For each cardiac outcome, a panel consisting of a consultant cardiologist, two geriatricians, and a general practitioner reviewed every potential events for adjudication of the final diagnosis.

Cerebrovascular disease included stroke and transient ischaemic attacks. Stroke was defined according to World Health Organisation criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting ≥24 hours or leading to death and no apparent cause other than of vascular origin.[14] We defined TIA as temporary attacks with presence of focal symptoms, which are attributable to dysfunction of

one arterial territory of the brain. Of all potential TIAs and strokes, medical records from general practitioners and hospital discharge letters were collected and reviewed by research physicians, after which a consensus panel headed by an experienced vascular neurologist decided on the diagnosis.

All researchers partaking in case ascertainment were blinded to participants' amyloid status. Twenty-year follow-up was virtually complete for all outcomes (99.4% of potential person years for dementia, 99.4% for cerebrovascular disease, and 96.0% for myocardial infarction). Longitudinal data for myocardial revascularisation procedures were not available.

Mortality coding

Information on vital status was obtained through automated linkage of general practitioner files with the study database, as well as a bimonthly check of municipal records. Information about cause and circumstances of death was obtained from general practitioner, nursing home, and hospital records. Research physicians, blinded to amyloid measurements, reviewed all available information, after which a consensus panel led by a consultant physician with cardiovascular expertise adjudicated the final cause of death according to the International Classification of Diseases, 10th edition (ICD-10).[14] ICD-10 codes that were considered cardiovascular causes of death included I20–25, I46, I50, I61, I63, I64, I66, I68–70, and R96. Follow-up for mortality until January 2014 was near-complete (99.7% of potential person years), although in 133 (7.3%) of deceased individuals no cause of death could be specified (ICD R99).

Quantification of atherosclerosis

For measurement of carotid intima-media thickness and atherosclerotic plaques, ultrasonography of both carotid arteries was performed with a 7.5-MHz linear-array transducer (ATL UltraMark IV, Advanced Technology Laboratories, Bothell, WA). A longitudinal image was obtained of the lumen-intima interface and the media-adventitia interface of the far wall of the distal common carotid artery, frozen on the R wave of the ECG, and stored for off-line measurement of intima-media thickness.[15] With a cursor the interfaces of the distal common carotid artery were marked over a length of 10mm, taking the beginning of the dilatation of the distal common carotid artery as reference point. The average of the intima-media thickness of three captured images was calculated, and the mean of the right and left carotid artery were used for analysis. Presence of atherosclerotic plaques, defined as a focal widening of the vessel wall relative to adjacent segments with protrusion into the lumen, was assessed in the common carotid arteries, the bifurcation of the carotid arteries, and the internal carotid arteries.[16]

Other measurements

At baseline, we assessed smoking status (i.e. current, former, never), and use of antihypertensive, lipid-lowering, and antithrombotic medication by interview. Non-fasting serum total cholesterol and high-density lipoprotein (HDL) cholesterol, haemoglobin levels, and estimated glomerular filtration rate (eGFR) were measured at baseline. Systolic and diastolic blood pressures were measured twice on the right arm with a random-zero sphygmomanometer; the mean of two readings was used for analysis. Body mass index (BMI) was computed from measurements of height and weight (kg/m^2). Diabetes was defined as the use of blood glucose-lowering medication at baseline, or a random serum glucose level ≥11.1 mmol/L. *APOE* genotype was determined using polymerase chain reaction on coded DNA samples.

Analysis

Of 1,834 eligible participants, we excluded 19 (1.0%) because of unreliable measurements of plasma amyloid (>3.5 standard deviations from the natural log transformed mean), thus leaving 1,815 participants for analysis. Missing covariate data (24.2% for GFR and <6.4% for all other covariates) were imputed using fivefold multiple imputation. Distribution of covariates was similar in the imputed versus non-imputed dataset.

We first assessed the correlation between Aβ40 and Aβ42 (Pearson r = 0.69). We subsequently verified that this correlation did not induce substantial collinearity in the model by calculating the variance inflation factor (VIF). The VIF was 1.9, meaning that the standard error for the coefficient of Aβ40 and Aβ42 is √1.9 = 1.4 times as large as it would be if these were uncorrelated with each other, which is generally considered minor.[17]

To investigate shared aetiological factors between coronary heart disease, cerebrovascular disease, and dementia, we then determined the association of plasma amyloid with cardiovascular and several other risk factors shared between dementia and vascular disease, using univariable and multivariable linear regression models. We verified that associations of BMI and haemoglobin with amyloid did not deviate from linearity by adding quadratic terms to the model. The analyses were repeated after exclusion of prevalent dementia cases.

Next, we determined the association of plasma amyloid levels with prevalent and incident coronary heart disease, cerebrovascular disease, and mortality, and for comparison also with dementia, using logistic regression and Cox regression models as appropriate. Twenty-one individuals withdrew consent for data collection during follow-up, and were excluded from the longitudinal analyses. As estimates for all-cause dementia and Alzheimer's disease only were very similar (data not shown), we present all-cause dementia among the main results.

Participants were censored within the follow-up period at date of event diagnosis, date of death, date of loss to follow-up, or administrative censoring date (at t=20 years), whichever came first. Incident analyses were furthermore time-stratified to account for non-proportionality of hazard functions over time. All analyses were adjusted for age, sex, and Aβ40 or Aβ42, and additionally in a second model for smoking habits, systolic and diastolic blood pressure, use of antihypertensive medication, serum total and HDL cholesterol, use of lipid-lowering medication, diabetes, BMI, use of antithrombotic medication, haemoglobin, renal function, and *APOE* genotype. To assess associations independent of dementia, we repeated analyses after excluding individuals with dementia at baseline, and censoring at time of dementia diagnosis during follow-up. We also assessed effect modification by age or sex by adding multiplicative interaction terms to the Cox models.

Finally, we determined the associations of Aβ40 or Aβ42 with carotid intima-media thickness using linear regression, and with carotid plaque score using analysis of covariance (ANCOVA) and ordinal regression, including in the model age, sex, Aβ40, and Aβ42.

All analyses were done using IBM SPSS Statistics version 21.0 (IBM Corp, Armonk, NY, USA). Alpha (type 1 error) was set at 0.05.

Characteristics	**Subcohort (N=1815)**	**Overall cohort (N=7983)**
Age	69.1 (±9.0)	70.6 (±9.8)
Female sex	1122 (61.8%)	4878 (61.1%)
Systolic blood pressure, mmHg	138 (±22)	139 (±22)
Diastolic blood pressure, mmHg	73 (±11)	74 (±12)
Antihypertensive medication	560 (30.9%)	2643 (33.2%)
Diabetes	114 (6.7%)	596 (8.9%)
Body-mass index	26.2 (±3.7)	26.3 (±3.7)
Serum cholesterol, mmol/L	6.7 (±1.2)	6.6 (±1.2)
Serum high-density lipoprotein, mmol/L	1.4 (±0.4)	1.3 (±0.4)
Lipid-lowering medication	36 (2.0%)	175 (2.2%)
Smoking		
Former	708 (40.8%)	2994 (40.1%)
Current	374 (21.5%)	1559 (20.9%)
Antithrombotic medication	95 (5.2%)	427 (5.4%)
Glomerular filtration rate, mL/min/1.73m^2	74 (±16)	74 (±16)
Haemoglobin, mmol/L	8.7 (±0.9)	8.7 (±0.9)
APOE genotype		
ε3/ε3	1038 (59.2%)	3990 (58.2%)
ε2/ε2, or ε2/ε3	225 (12.8%)	928 (13.5%)
ε2/ε4, ε3/ε4, or ε4/ε4	489 (27.9%)	1935 (28.2%)
Amyloid-β40, pg/mL (median, IQR)	194 (164-231)	n/a
Amyloid-β42, pg/mL (median, IQR)	17.9 (14.7-21.7)	n/a

Table 1. Baseline characteristics. Data are presented as frequency (%) for categorical, and mean±standard deviation for continuous variables unless indicated otherwise. IQR=interquartile range.

RESULTS

Baseline characteristics of participants are presented in Table 1, including a comparison of this random subcohort with the full Rotterdam Study cohort. Participants were on average slightly younger than the overall cohort, but similar in every other measured aspect.

Plasma amyloid levels were moderately correlated with age (r=0.39 for Aβ40, and r=0.23 for Aβ42; Table 2). Consistent with dementia risks, *APOE* ε4 carriers had higher Aβ40 and lower Aβ42, and *APOE* ε2 carriers tended to have lower Aβ40 and higher Aβ42. In multivariable models, high HDL cholesterol was associated with lower levels of Aβ40 and Aβ42, whereas high total cholesterol was associated with increased levels of Aβ42. Aβ40 and Aβ42 were higher with impaired renal function, and Aβ40, but not Aβ42, was higher in women and in participants with anaemia. These associations were all broadly unaltered after exclusion of prevalent dementia cases (data not shown).

At baseline, 78 individuals had dementia (52 Alzheimer's disease), 131 coronary heart disease (102 myocardial infarction), and 95 cerebrovascular disease (54 stroke and 49 TIA). Higher levels of plasma Aβ40 were associated with presence of each manifestation of vascular disease, with effect estimates comparable, albeit a little lower than for dementia (Table 3). Conversely, lower levels of plasma Aβ42 were associated with prevalent dementia, but not coronary heart disease or cerebrovascular disease (Table 3). Additional adjustment for cardiovascular risk factors and *APOE* genotype somewhat attenuated estimates of Aβ40 for coronary heart disease, but did not substantially alter any of the other associations (Table 3). Associations were unaltered by excluding individuals with dementia (data not shown). Similar results were observed when segregating cerebrovascular disease by stroke and TIA. When assessing Aβ40 and Aβ42 in separate models, rather than mutually adjusted, estimates for Aβ40 remained similar, but those of Aβ42 were more similar to Aβ40 (Table 4).

	Dementia (n/N=78/1815) OR (95% CI)	**Cerebrovascular disease (n/N=95/1815)** OR (95% CI)	**Coronary heart disease (n/N=131/1677)** OR (95% CI)
Model I			
Amyloid β-40	1.98 (1.33-2.96)	1.60 (1.17-2.19)	1.45 (1.11-1.91)
Amyloid β-42	0.52 (0.34-0.79)	0.91 (0.65-1.28)	1.06 (0.80-1.41)
Model II			
Amyloid β-40	1.90 (1.24-2.92)	1.61 (1.17-2.23)	1.33 (0.98-1.80)
Amyloid β-42	0.54 (0.35-0.86)	0.87 (0.61-1.23)	0.87 (0.63-1.20)

Table 3. Amyloid and prevalent disease. Values reflect estimates per SD increase in plasma amyloid levels. Cerebrovascular disease includes stroke and TIA; Coronary heart disease includes myocardial infarction and coronary artery revascularisation. Model I is adjusted for age, sex, and β-40 or β-42; model II additionally includes all other factors from Table 1. SD=standard deviation; OR=odds ratio; CI=confidence interval.

	Amyloid β-40		Amyloid β-42	
	Univariable models† β (95% CI)	Multivariable model‡ β (95% CI)	Univariable models† β (95% CI)	Multivariable model‡ β (95% CI)
Age, per 10 years	**0.245 (-0.212;0.279)*****	**0.162 (0.114;0.209)*****	**-0.040 (-0.074;-0.006)***	**-0.060 (-0.108;-0.013)***
Female sex	**0.084 (0.020;0.148)****	**0.080 (0.006;0.155)***	0.014 (-0.045;0.073)	0.039 (-0.034;0.112)
Systolic blood pressure, per 10 mmHg	**0.019 (0.005;0.033)****	-0.004 (-0.022;0.014)	-0.002 (-0.015;0.011)	-0.010 (-0.028;0.008)
Diastolic blood pressure, per 10 mmHg	**-0.029 (-0.057;-0.001)***	-0.010 (-0.044;0.024)	0.018 (-0.008;0.044)	0.016 (-0.018;0.051)
Antihypertensive medication	**0.173 (0.106;0.240)*****	0.057 (-0.011;0.125)	0.024 (-0.039;0.087)	0.009 (-0.057;0.076)
Diabetes	**0.202 (0.072;0.333)****	0.013 (-0.106;0.132)	-0.041 (-0.159;0.077)	-0.085 (-0.208;0.037)
Body-mass index	-0.005 (-0.013;0.004)	**-0.010 (-0.019;-0.001)***	**0.011 (0.003;0.019)****	0.008 (-0.0002;0.017)
Serum cholesterol, per mmol/L	**-0.032 (-0.057;-0.007)***	-0.002 (-0.027;0.023)	**0.028 (0.005;0.051)***	**0.028 (0.004;0.052)***
Serum high-density lipoprotein, per mmol/L	**-0.143 (-0.224;-0.061)*****	**-0.180 (-0.262;-0.098)*****	**-0.088 (-0.164;-0.013)***	**-0.103 (-0.184;-0.022)***
Lipid-lowering medication	-0.153 (-0.380;0.073)	-0.153 (-0.366;0.059)	0.024 (-0.181;0.228)	0.032 (-0.176;0.241)
Smoking (compared with never smokers)				
Former	**-0.106 (-0.177;-0.035)****	0.016 (-0.056;0.088)	0.012 (-0.053;0.077)	0.027 (-0.044;0.098)
Current	**-0.167 (-0.252;-0.081)*****	-0.010 (-0.095;0.075)	0.055 (-0.023;0.133)	0.050 (-0.033;0.132)
Antithrombotic medication	**0.191 (0.052;0.330)****	0.072 (-0.063;0.207)	0.031 (-0.097;0.160)	0.022 (-0.110;0.155)
Haemoglobin, per mmol/L	**-0.101 (-0.137;-0.065)*****	**-0.062 (-0.104;-0.020)****	0.031 (-0.004;0.065)	0.015 (-0.025;0.054)
eGFR, per 10 mL/min/1.73m^2	**-0.127 (-0.147;-0.107)*****	**-0.067 (-0.097;-0.037)*****	-0.016 (-0.036;0.005)	**-0.043 (-0.077;-0.009)***
APOE genotype (compared with homozygous ε3)				
ε2/ε2, or ε2/ε3	-0.062 (-0.159;0.035)	-0.036 (-0.126;0.054)	0.072 (-0.019;0.162)	0.081 (-0.011;0.174)
ε2/ε4, ε3/ε4, or ε4/ε4	**0.100 (0.029;0.171)***	**0.104 (0.037;0.171)****	**-0.123 (-0.189;-0.057)*****	**-0.133 (-0.200;-0.066)*****

Table 2. Determinants of plasma amyloid levels. *0.05<*P*≥0.01; **0.01<*P*≥0.001; ***P<0.001; † adjusted for β-40 or β-42; ‡includes all variables presented in the table

	Dementia (n/N=304/1723) RR (95% CI)*	Cerebrovascular disease (n/N=357/1701) RR (95% CI)*	Myocardial infarction (n/N=141/1673) RR (95% CI)*
Prevalent disease			
Amyloid β-40	1.28 (0.97-1.70)	1.51 (1.20-1.91)	1.51 (1.22-1.85)
Amyloid β-42	0.86 (0.65-1.15)	1.27 (1.00-1.61)	1.36 (1.10-1.68)
Incident disease			
Amyloid β-40	0.97 (0.84-1.12)	0.94 (0.82-1.08)	1.07 (0.86-1.31)
Amyloid β-42	0.79 (0.68-0.92)	0.95 (0.83-1.08)	1.03 (0.83-1.27)

Table 4. Amyloid and cardiovascular disease without adjustment for the other isoform. Values reflect estimates per SD increase in amyloid levels. Model is adjusted for age and sex. *Odds ratio for prevalent disease; hazard ratio for incident disease. SD=standard deviation; RR=relative risk; CI=confidence interval.

During 25,175 person years of follow-up (median 16.5 years), 1,085 individuals died, of whom 376 (34.7%) due to cardiovascular causes. In the same follow-up period, 304 individuals developed dementia (227 Alzheimer's disease), 357 had cerebrovascular disease (240 stroke and 169 TIA), and 141 individuals had myocardial infarction.

Higher Aβ40 was associated with mortality, and in particular death due to cardiovascular causes (adjusted HR [95% CI] for all-cause mortality– 1.22 [1.11-1.35], and for cardiovascular mortality– HR 1.32 [1.11-1.56]). Risk estimates were highest in the first decade of follow-up, somewhat attenuating thereafter (Figure 1). For Aβ42, we observed no significant associations with mortality, regardless of the cause of death (Figure 1). The overall hazard ratio of 0.92 (95% CI 0.83-1.01) for Aβ42 further attenuated after exclusion of individuals with prevalent dementia and censoring at time of incident dementia diagnosis (HR 0.99 [0.88-1.11]). Associations with Aβ40 persisted after accounting for dementia, albeit slightly attenuated (HR 1.16 [1.03-1.31]).

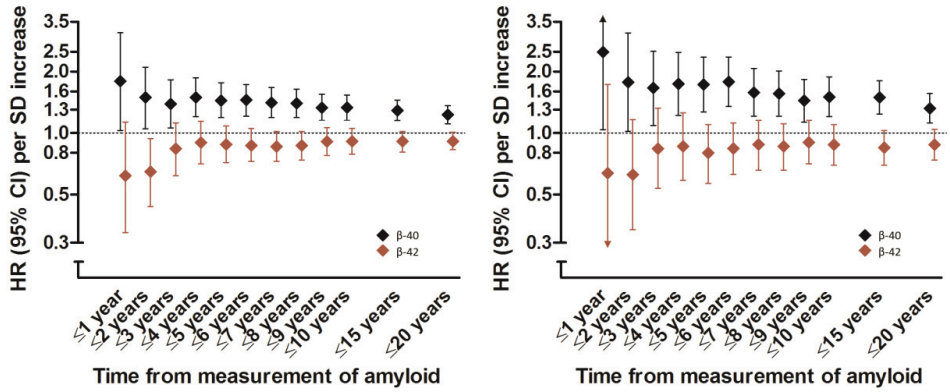


Figure 1. Time-stratified all-cause mortality (L), and cardiovascular mortality (R). HR=hazard ratio; CI=confidence interval; SD=standard deviation.

Observed patterns for risk of dementia and myocardial infarction were similar to those for mortality, showing elevated risks with higher Aβ40 that largely extended throughout the first decade of follow-up, but attenuated thereafter (Figure 2). Consequently, there were no overall, 20-year associations of Aβ40 with either risk of myocardial infarction or cerebrovascular disease (Table 5). This was again similar for stroke and TIA. In contrast to dementia, no associations were observed for Aβ42 with myocardial infarction or cerebrovascular disease (Table 3), regardless of follow-up duration. There was no evidence of effect modification by age or sex for any of the longitudinal outcome measures (all *P*-values for interaction ≥0.51). Among participants in whom atherosclerotic burden was quantified, neither Aβ40 nor Aβ42 were related to carotid intima media thickness or the number of atherosclerotic plaques in the carotid artery (Table 6).

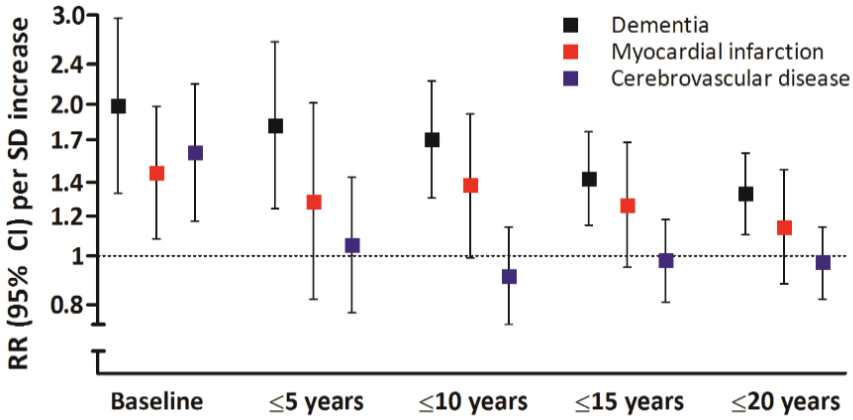


Figure 2 Plasma amyloid-β40 and risk of vascular disease in comparison to dementia risk, stratified by follow-up time. Relative risks are odds ratio for prevalent disease, and hazard ratios for incident disease. RR=relative risk; CI=confidence interval; SD=standard deviation.

	Dementia (n/N=304/1723) HR (95% CI)	**Cerebrovascular disease (n/N=357/1701)** HR (95% CI)	**Myocardial infarction (n/N=141/1673)** HR (95% CI)
Model I			
Amyloid β-40	1.33 (1.10-1.60)	0.97 (0.82-1.14)	1.14 (0.88-1.48)
Amyloid β-42	0.64 (0.53-0.78)	1.01 (0.85-1.20)	1.05 (0.81-1.36)
Model II			
Amyloid β-40	1.23 (1.02-1.49)	0.96 (0.80-1-14)	1.08 (0.83-1.41)
Amyloid β-42	0.69 (0.56-0.84)	0.98 (0.82-1.16)	0.97 (0.74-1.28)

Table 5. Amyloid and incident disease during the entire 20-year follow-up. Values reflect estimates per SD increase in plasma amyloid levels. Model I includes age, sex, and β-40 or β-42; model II additionally includes all other factors from Table 1. SD=standard deviation; HR=hazard ratio; CI=confidence interval

	Plasma amyloid levels by plaque count (mean, standard error)					Regression model (OR, 95% CI)
Plaque count (n=1428)	**0**	**1**	**2**	**3**	**≥4**	
Amyloid β-40 (pg/mL)	201.0 (1.8)	205.7 (2.9)	202.4 (2.6)	197.6 (3.8)	198.9 (2.8)	-0.064 (-0.214;0.085)
Amyloid β-42 (pg/mL)	18.9 (0.2)	18.8 (0.3)	19.1 (0.3)	19.0 (0.4)	19.3 (0.3)	0.116 (-0.039;0.272)
IMT quartiles (n=1420)		**Q1**	**Q2**	**Q3**	**Q4**	
Amyloid β-40 (pg/mL)	-	202.9 (2.4)	201.6 (2.3)	195.9 (2.2)	195.6 (2.3)	-0.032 (-0.102;0.039)
Amyloid β-42 (pg/mL)	-	18.9 (0.3)	18.6 (0.3)	19.2 (0.3)	18.7 (0.3)	0.017 (-0.057;0.090)

Table 6. Plasma amyloid and atherosclerotic plaque burden in the carotid artery. Estimates from ordinal (plaque score) and linear (IMT) regression are per standard deviation increase in plasma amyloid levels. Models are adjusted for age, sex, and Aβ-40 or Aβ-42. OR=odds ratio; CI=confidence interval.

DISCUSSION

In this prospective population-based study, higher plasma levels of amyloid-β40 are associated with presence of coronary heart disease and cerebrovascular disease, along with an increased risk of cardiovascular mortality, consistent with risk estimates for dementia. In contrast, amyloid-β42 was strongly associated with dementia, but not with manifestations of coronary heart disease or cerebrovascular disease. These findings imply a role of Aβ40 in atherosclerotic cardiovascular events, and suggest that various length amyloid peptides may be involved in different pathways culminating in atherosclerosis and/or neurodegeneration.

Given the large contribution of vascular disease to dementia, including clinical Alzheimer's disease,[1,2] and the uncertainty about the underlying mechanisms, remarkably few studies have assessed classical hallmarks of Alzheimer's disease pathology in relation to vascular disease. Our findings are in line with previous studies showing cross-sectional associations of Aβ40 with coronary artery disease in non-demented elderly,[8] and with ischaemic heart disease in the Swedish BioFINDER study.[4] Findings for Aβ42 in the same two studies were conflicting,[4,8] potentially due to lack of adjustment for levels of Aβ40 in the latter which may have induced a spurious association (Table 4). Distinct roles of the isoforms are supported by findings from the French Three-City study, in which Aβ40, but not Aβ42, was associated with mortality risk.[10] Similar increases in cardiovascular mortality with high Aβ40 were reported in patients with coronary heart disease.[9] Importantly, we confirmed that these associations are robust to adjustment for a wide range of potential confounders, and are independent of dementia diagnosis. Further study remains nevertheless required to determine to what extent changes in plasma Aβ40 occur systemically, independent of production or drainage

from the central nervous system, or because of a shift of Aβ40 from the central nervous system into the systemic circulation with increased blood-brain barrier permeability due to small-vessel disease.

The absence of associations with Aβ42, in stark contrast with observations for dementia, suggests that separate mechanisms underlie changes in isoform levels with cardiovascular disease and dementia. This has previously been noted in the context of cerebral amyloid angiopathy. Of different genetic forms of cerebral amyloid angiopathy, those with increases in Aβ40 seem to lead to increased amyloid deposition in the vessel walls, whereas increase in Aβ42 tend to result in parenchymal amyloid.[18,19] While cerebral amyloid angiopathy generally manifests exclusively in the central nervous system, our findings imply that plasma Aβ40 can mark more generalised vascular disease. Given the presence of Aβ40 in the vessel wall, endothelial and tunica media alterations could be the substrate.[20,21] Associations of Aβ40 with pulse wave velocity and intima-media thickness in younger, healthy individuals indeed suggest a role of Aβ40 in the process of atherosclerosis,[9] but these findings did not replicate in our sample. Alternative explanations include arteriolosclerosis, as evidenced by increased Aβ40 with cerebral small-vessel disease,[22] and a role of amyloid in the innate immune system,[23] as well as a modulator of inflammatory response.[24-26] In this context, Aβ40 may reflect macrophage activation within plaques and plaque instability rather than presence of atherosclerosis itself. Future studies will have to determine whether Aβ40 indeed relates to (subclinical) morphological changes in the arterial vessel wall. Additional studies with repeated measures of plasma amyloid-β could furthermore be useful to map changes in amyloid levels over time in relation to development of vascular and neurodegenerative disease.

Identifying determinants of plasma amyloid-β levels can avoid confounding in future studies, and add insight to the largely unknown physiological function of amyloid-β. Changes in plasma amyloid levels mirroring dementia risk are consistently seen with *APOE* genotype,(8) age,[8,9] and sex (Aβ40 only),[8,9] whereas previously reported associations with diabetes,[8] and hypertension[4] did not persist in our study after multivariable adjustment. We additionally found associations with body-mass index, total cholesterol (Aβ42), and haemoglobin (Aβ40) similar to dementia risk. Higher HDL cholesterol and better renal function related to lower levels of both Aβ40 and Aβ42,[9] suggesting these associations are driven by cholesterol transport (e.g. unmeasured lipid fractions) and amyloid clearance from the bloodstream, respectively.[27]

There are certain limitations to our study to take into account. First, contemporary assays of plasma amyloid are more sensitive and precise than the initial ELISA we used in this study,[28]

and novel measuring methods may consequently reduce measurement error. Second, we had no measurements of amyloid within the central nervous system, and while correlations are generally low,[4] additional studies with comparison of central and peripheral levels are needed to determine similarities and discrepancies in the pathophysiology of neurodegeneration and cardiovascular disease. Third, we did not measure amyloid peptides beyond those of 40 and 42 amino acids long, such as Aβ38, which may relate to both levels of β40, β42, and vascular risk.[22] Of note, estimates for Aβ42 varied widely in our models depending on whether amyloid-β40 was included, emphasising the importance of investigating effects of the separate peptides independent of one another. Fourth, no data were available about further aetiological subtyping of stroke, beyond ischaemia versus haemorrhage, which may yield different results in light of cerebral amyloid angiopathy and small-vessel pathology. Fifth, we only measured atherosclerosis in the carotid artery, and despite reasonable to good reproducibility,[15,29] (concurrent) measurements at other sites or using contemporary imaging modalities may more reliably determine how Aβ40 relates to atherosclerosis.

In conclusion, higher plasma levels of Aβ40, but not Aβ42, are associated with presence of cardiovascular disease and mortality risk. These findings suggest differential roles of various length amyloid peptides in cardiovascular disease, warranting further study of central nervous system and systemic amyloid-β isoforms to elucidate their role in CHD and stroke, as well as dementia.

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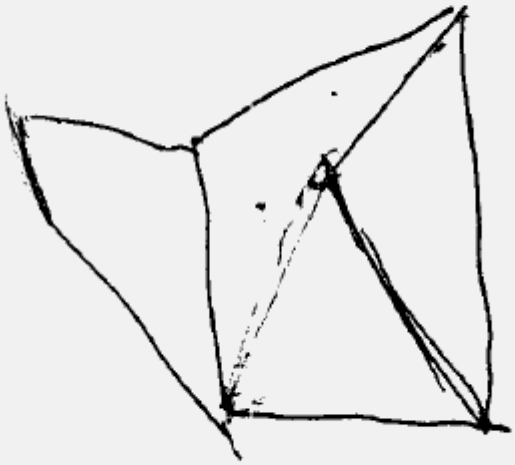
Chapter 4.4

Von Willebrand factor and ADAMTS13



ABSTRACT

Low ADAMTS13 activity is associated with an increased risk of cardiovascular disease, which is generally attributed to its proteolytic effects on Von Willebrand factor (VWF). Cardiovascular health is an important determinant of cognitive decline, but the association of either VWF or ADAMTS13 with risk of dementia is unknown. Between 1997-2002, we measured VWF antigen and ADAMTS13 activity in 6055 participants of the population-based Rotterdam Study (mean age 69.3 years, 57.2% women). At baseline, 85 participants had dementia, and during 15 years of follow-up 821 developed dementia. Higher VWF was associated with prevalence and risk of dementia, unaffected by concurrent ADAMTS13 activity, but estimates strongly attenuated over time and were no longer statistically significant at 4 years of follow-up (relative risks [95%CI] per standard deviation increase– cross-sectional: 1.37 [1.06-1.77], and longitudinal: 1.05 [0.97-1.14]). In contrast, low ADAMTS13 was associated with increased risk of dementia throughout follow-up (hazard ratio per SD decrease– 1.16 [1.06-1.28]), which alike for ischaemic stroke, was modified by the presence of diabetes (*P*-interaction=0.003). In conclusion, higher VWF and low ADAMTS13 activity are associated with increased risk of dementia, but differences in time-course and lack of synergistic effects may indicate in part independent underlying mechanisms.

INTRODUCTION

Von Willebrand factor (VWF) is a large multimeric glycoprotein with critical functions in haemostasis. Deficiency or dysfunction of VWF, known as Von Willebrand disease, can cause prolonged or excessive bleeding,[1] whereas high levels of VWF antigen have been associated with increased risk of cardiovascular disease.[2] In vivo effects of VWF largely depend on the proteolytic activity of ADAMTS13 (A Disintegrin And Metalloproteinase with a ThromboSpondin type 1 motif, member 13). ADAMTS13 cleaves large, haemostatically highly reactive VWF multimers into smaller, less active multimers. Consequently, high VWF may lead to a hypercoagulable state in particular when ADAMTS13 activity is low, and a combined measure of VWF and ADAMTS13 could thus more accurately capture the biological activity of VWF.[3] We have previously shown that low activity of ADAMTS13 itself is associated with increased risk of cardiovascular disease,[4-8] while the combination of VWF and ADAMTS13 appears indeed more strongly associated with stroke risk than what would be expected on the basis of the individual measurements.[5]

Vascular disease and thrombosis play an important role in the aetiology of dementia, including Alzheimer's disease.[9] Accordingly, a recent meta-analysis of cross-sectional studies concluded that VWF antigen levels are higher in patients with dementia than in controls.[10] However, of two studies that assessed the risk of dementia by VWF,[11,12] neither found baseline VWF antigen levels associated with dementia risk after 4 and 17 years of follow-up, respectively, albeit the latter was hampered by substantial attrition (50%) and lack of cognitive screening at baseline. Apart from methodological considerations, release of VWF from damaged endothelial cells in later stages of cognitive impairment may explain why profound cross-sectional associations do not extend to longer term follow-up. However, the time-course of the association between VWF and dementia remains unknown, and although ADAMTS13 could aid in disentangling haemostatic effects from associations marking endothelial damage, no published studies about VWF and dementia took into account concurrent ADAMTS13 activity.

While VWF is the only known substrate for ADAMTS13, several studies suggest that ADAMTS13 might have functions beyond VWF cleavage. Suggested roles include inflammation, angiogenesis, and extracellular matrix integrity,[13] each of which have been implicated also in the aetiology of dementia.[14-16] A versatile role of ADAMTS13 was further suggested, when we recently showed that *high* activity of ADAMTS13 relates to a *higher* risk of diabetes in the general population.[17] The underlying mechanisms remain elusive, but these studies jointly highlight the need for investigation of ADAMTS13 in the context of, as well as beyond its proteolytic activity of VWF.

We aimed to determine the cross-sectional and long-term associations of VWF and ADAMTS13 with cognitive decline and dementia risk in a population-based study. We investigated independent and synergistic effects of VWF and ADAMTS13, and explored these associations in the context of prior studies linking ADAMTS13 to diabetes, angiogenesis, and extracellular matrix integrity.

METHODS

Study population

This study is part of the Rotterdam Study, a large ongoing population-based cohort study in the Netherlands, with an initial study population of 7,983 participants aged ≥55 years from the Ommoord area, a suburb of Rotterdam. In 2000, the cohort was expanded with an additional 3011 participants who moved into the study area or reached age 55. The Rotterdam Study methods have been described previously.[18] Briefly, participants were interviewed at home and subsequently examined at the research centre for baseline assessment from 1990 to 1993 (baseline cohort) and 2000 to 2002 (expansion cohort), with follow-up examinations every 4 years. Citrated plasma samples were collected at the third visit of the original cohort (1997-1999), and the first visit of the expansion cohort (2000-2002), which are the baseline of the current study. Of 9,030 surviving participants at the time, 7,510 participated in this examination cycle, of whom 6,735 visited the study centre. Of these, 43 had insufficient cognitive screening to determine dementia status.

Measurement of Von Willebrand factor antigen and ADAMTS13 activity

Fasting venous blood samples were taken at the research centre, and citrated plasma was stored at –80°C. We determined VWF antigen with an in-house enzyme-linked immunosorbent assay using polyclonal rabbit antihuman VWF antibodies (DakoCytomation, Glostrop, Denmark) for catching and tagging. The intra-assay coefficient of variation was 5.8% and the inter-assay coefficient of variation was 7.8%. We measured ADAMTS13 activity using a kinetic assay based on the fluorescence resonance energy transfer substrate VWF73 (FRETSVWF73) assay.[19] This assay uses a peptide containing the ADAMTS13 cleavage site of VWF, and thus captures variation in the VWF cleavage rate determined by ADAMTS13 levels and structure. Plasma samples were measured against a reference curve of serial dilutions of normal human plasma defined to have an ADAMTS13 activity of 1 IU/mL, and we expressed ADAMTS13 activity as a percentage of this. Ten percent of the samples were retested and all were within 25% variation. From these measurements, we also calculated the ratio between ADAMTS13 activity and VWF antigen levels.

Cognitive function assessment

Participants underwent detailed tests to determine cognitive function, comprising the Stroop test (error adjusted time in seconds taken for completing a reading/colour naming interference task), the letter-digit substitution task (number of correct digits in 1 minute), and the verbal fluency test (number of animal species within 1 minute).[20] Cognitive function was assessed at baseline (i.e. time of blood sampling) and at three subsequent follow-up examinations (after a mean follow-up of 4.4 (SD 0.6), 10.8 (SD 0.6), and 15.4 (SD 0.7) years, respectively). To obtain a composite measure of test performance, we calculated the *g*-factor, which explained approximately 61% of variance in cognitive test scores at each examination round in our population. For each participant, Z-scores were calculated for each test separately, by dividing the difference between individual test score and population mean by the population standard deviation.

Dementia screening and surveillance

Participants were screened for dementia at baseline and subsequent centre visits using the Mini-Mental State Examination (MMSE) and the Geriatric Mental State Schedule (GMS) organic level.[21] Those with MMSE<26 or GMS>0 underwent further investigation and informant interview including the Cambridge Examination for Mental Disorders of the Elderly. Additionally, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study centre with medical records from general practitioners and the regional institute for outpatient mental healthcare. Available clinical neuroimaging data were reviewed when required for diagnosis of dementia subtype. A consensus panel led by a consultant neurologist established the final diagnosis according to standard criteria for dementia (DSM-III-R), and Alzheimer's disease (NINCDS-ADRDA).

Measurement of other blood markers

In a subset of 1,075 non-demented participants, we measured at baseline 150 plasma markers via multiplex immunoassay on human multianalyte profiles in the fasting blood samples collected at baseline (Myriad RBM Inc., Austin TX, USA; http://rbm.myriad.com). Of these, we selected markers with an identified role in angiogenesis (i.e. angiopoietin-2 (ANG-2), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factors α and β (TGF-α, TGF-β)) or related to the extracellular matrix (i.e. matrix metalloproteinases MMP-2, MMP-3, and MMP-9, tissue inhibitor of metalloproteinase-1 (TIMP-1), Tenascin-C, connective tissue growth factor (CTGF)), based on suggested roles of ADAMTS13 beyond regulation of thrombosis.(13) The assay did not pass quality control (>20% unmeasurable) for TGF-α, TGF-β, MMP-2, MMP-9 and CTGF, leaving 6 markers for analysis (all with measurements in ≥92.4% of participants).

Other measurements

We assessed smoking habits and use of antihypertensive, lipid-lowering, glucose lowering, and antithrombotic (i.e. coumarine derivatives or platelet inhibitors) medication at baseline by interview. Blood pressure was measured with a random-zero sphygmomanometer. Fasting serum lipid levels, C-reactive protein (CRP) and fibrinogen were measured at baseline. Diabetes, prediabetes and normoglycaemia were defined according to WHO guidelines.[22] *APOE* genotype was determined using polymerase chain reaction on coded DNA samples (baseline cohort), or using a bi-allelic TaqMan assay (rs7412 and rs429358; expansion cohort). ABO blood group antigen phenotypes were reconstructed by haplotype analysis of single nucleotide polymorphisms (rs8176749, rs8176704, and rs505922), and classified into O and non-O. We assessed history of stroke and myocardial infarction by interview, consultation of medical records, and electrocardiography.

Analysis

Because of a right-skewed distribution of VWF and the ADAMTS13:VWF-ratio, we performed a natural logarithmic transformation to obtain a roughly normal distribution of the data. We computed Z-scores for each individual by dividing the difference between the individual value and the population mean by the population standard deviation.

Missing covariate data (15.0% for ABO blood type, and <5.0% for all other covariates) were imputed using fivefold multiple imputation. Distribution of covariates was similar in the imputed versus non-imputed dataset. All analyses were adjusted for age, sex, and study subcohort. In a second model we further adjusted for systolic and diastolic blood pressure, use of antihypertensive medication, serum total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides, use of lipid-lowering medication, body mass index, diabetes, creatinine, CRP, fibrinogen, ABO blood type, and use of antithrombotic medication.

We determined the association of VWF and ADAMTS13 with prevalence and incidence of dementia, using logistic regression and Cox proportional hazard models, respectively. As the proportional hazard assumption was violated for VWF, we also determined associations with dementia risk per year increase in follow-up. We determined risk of dementia per standard deviation (SD) increase as well as per quartile of VWF, ADAMTS13, and their ratio. In view of previously suggested threshold effects of ADAMTS13, we also compared the lowest quartile of ADAMTS13 to the highest three quartiles altogether.[2] We assessed effect modification by (pre-)diabetes, by testing for multiplicative interaction in the fully adjusted Cox model. We repeated the analysis after excluding participants with prevalent myocardial infarction or stroke, while censoring at time of incident myocardial infarction or stroke in the fully adjusted model. We performed further sensitivity analyses, 1) for Alzheimer's disease only,

2) stratifying by the mean age of the study population (i.e. 69.3 years), 4) stratifying by sex, and 5) stratifying by blood type O versus non-O.

We then determined the association of VWF levels and ADAMTS13 activity with change in scores on cognitive assessment during follow-up, using linear mixed models. We fitted a model (restricted maximum likelihood) to the *g*-factor of cognitive scores, including age, sex, follow-up time, time*age, VWF/ADAMTS13, and time*VWF/ADAMTS13 in the model. We chose a diagonal covariance structure (heterogeneous variance and zero correlation between elements) for the random effects, including a random intercept and follow-up time, and added other covariates in agreement with the fully adjusted model described above. We repeated the analysis for all cognitive tests, stratified by diabetic status, and limited to the 1st, 2nd, and 3rd follow-up examination, respectively.

Finally, in the subset of participants with immunoassay data, we determined correlations of ADAMTS13 with ANG-2, VEGF, PDGF, MMP-3, TIMP-1, and Tenascin-C, using linear regression (of natural log-transformed values if so required to obtain normal distributions of the data). Values exceeding ±3.5 standard deviations from the mean were excluded. We fitted univariable models, and additional models including age, sex, and each of the other biomarkers, whilst applying the Benjamini-Hochberg correction for multiple testing.

All analyses were done using SPSS Statistics 21.0 (IBM Corp, Armonk, NY, USA) or R statistical software 3.1.1 (package 'nlme'). Alpha level was set at 0.05.

RESULTS

Among 6,692 eligible participants, we could not determine VWF antigen in 380 participants and ADAMTS13 activity in 628 participants, mainly due to technical reasons or insufficient blood sampling, leaving 6055 (90.5%) participants with both measures for analyses. Baseline characteristics of the study population are presented in Table 1.

At baseline, 85 participants had dementia, of whom 68 had Alzheimer's disease. Participants with dementia had higher VWF antigen levels and lower ADAMTS13 activity than individuals without dementia (Table 2). Consequently, the ADAMTS13:VWF ratio was lower in individuals with dementia, but ADAMTS13 did not modify the association of VWF with dementia (Table 2; *P*-interaction=0.93), and adjustment for ADAMTS13 did not change VWF estimates. Associations of VWF and ADAMTS13 with dementia were mildly attenuated for Alzheimer's disease only, and broadly unaltered by excluding cardiovascular disease.

Characteristics	Study population
Age, years	69.3 (±8.2)
Women	3,461 (57.2)
Systolic blood pressure, mmHg	143 (±21)
Diastolic blood pressure, mmHg	77 (±11)
Antihypertensive medication	2,017 (35.0)
Pre-diabetes	1,663 (28.1)
Diabetes	744 (12.6)
Serum cholesterol, mmol/L	5.82 (±0.98)
Serum HDL cholesterol, mmol/L	1.39 (±0.39)
Serum triglycerides, mmol/L (median, IQR)	1.35 (1.03-1.81)
Lipid-lowering medication	746 (12.8)
Smoking	
Former	2,958 (49.3)
Current	1,032 (17.2)
Creatinine, mg/dL	0.89 (±0.21)
Body-mass index, kg/m^2	26.9 (±4.0)
History of cardiovascular disease	283 (4.7)
Anti-thrombotic medication	1,135 (18.7)
APOE genotype	
ε3/3	3,389 (58.1)
ε2/2 or ε2/3	821 (14.1)
ε2/4 or ε3/4, ε4/4	1,627 (27.9)
Von Willebrand factor, IU/mL (median, IQR)	1.20 (0.93-1.60)
ADAMTS13, %	91.5 (±17.7)
Fibrinogen, g/L (median, IQR)	3.8 (3.3-4.4)
C-reactive protein, mg/mL (median, IQR)	1.8 (0.7-3.7)
Blood type O	2348 (45.6)

Table 1. Baseline characteristics of the 6,055 participants. Data are presented as frequency (%) for categorical, and mean±standard deviation for continuous variables, unless indicated otherwise; IQR=interquartile range.

Of 5,970 non-demented participants at baseline, 821 participants were diagnosed with dementia during a mean follow-up of 11.6 years (follow-up was complete for 97.5% of potential person years). Of all dementia diagnoses, 671 were due to Alzheimer's disease, and 154 were preceded by myocardial infarction or a stroke. At baseline, 5,844/5,970 (97.9%) participants underwent extensive cognitive assessment, of whom 4,582 (78.4%) underwent at least two assessments, and 2,934 (50.2%) attended at least three examinations.

Overall, VWF antigen levels were not associated with risk of dementia (adjusted HR per SD increase: 1.05, 0.97-1.14). VWF levels were, however, associated with short-term risk of dementia, but these associations attenuated over time and were no longer statistically significant beyond 4 years of follow-up (Figure 1A). Similarly, associations of VWF with cognitive test performance at baseline extended to the first re-examination at 4.4 years, but not thereafter (Figure 1B). The associations of VWF with cognitive decline and risk of dementia were not affected by concurrent ADAMTS13 activity (*P*-value for interaction of VWF with ADAMTS13 = 0.58 for all-cause dementia, and 0.85 for the *g*-factor).

	All-cause dementia (Model I)			All-cause dementia (Model II)		
	No (*N*=5,970)	Yes (*N*=85)	*P*-value	No (*N*=5,970)	Yes (*N*=85)	*P*-value
VWF:Ag						
Geometric mean (95% CI)	1.22 (1.20-1.23)	1.34 (1.23-1.46)	0.021	1.23 (1.20-1.26)	1.36 (1.26-1.48)	0.013
OR (95% CI) per SD increase	1.29 (1.04-1.62)		0.023	1.37 (1.06-1.77)		0.017
ADAMTS13 activity						
Mean (95% CI)	91.2 (90.7-91.6)	86.1 (82.5-89.7)	0.007	91.2 (90.1-92.4)	86.9 (83.2-90.6)	0.015
OR (95% CI) per SD decrease	1.28 (1.01-1.64)		0.046	1.25 (0.95-1.63)		0.107
ADAMTS13:VWF ratio						
Geometric mean (95% CI)	73.5 (72.6-74.3)	61.9 (56.3-68.2)	0.001	72.5 (70.5-74.7)	61.6 (56.1-67.6)	0.0004
OR (95% CI) per SD decrease	1.39 (1.12-1.72)		0.003	1.44 (1.13-1.85)		0.004

Table 2 Von Willebrand factor (VWF) and ADAMTS13 at baseline in relation to the prevalence of dementia. Model I is adjusted for age, sex, study subcohort. Model II is additionally adjusted for smoking, systolic and diastolic blood pressure, antihypertensive medication, diabetes, serum cholesterol, high density lipoprotein cholesterol and triglycerides, lipid-lowering medication, body mass index, creatinine, antithrombotic medication, fibrinogen, C-reactive protein, ABO blood type, and *APOE* genotype. N=number of participants; SD=standard deviation; OR=odds ratio from logistic regression model; CI=confidence interval. Geometric means facilitate a comparison of normalized results, as is the case for the not normally distributed VWF and the ADAMTS13:VWF ratio.

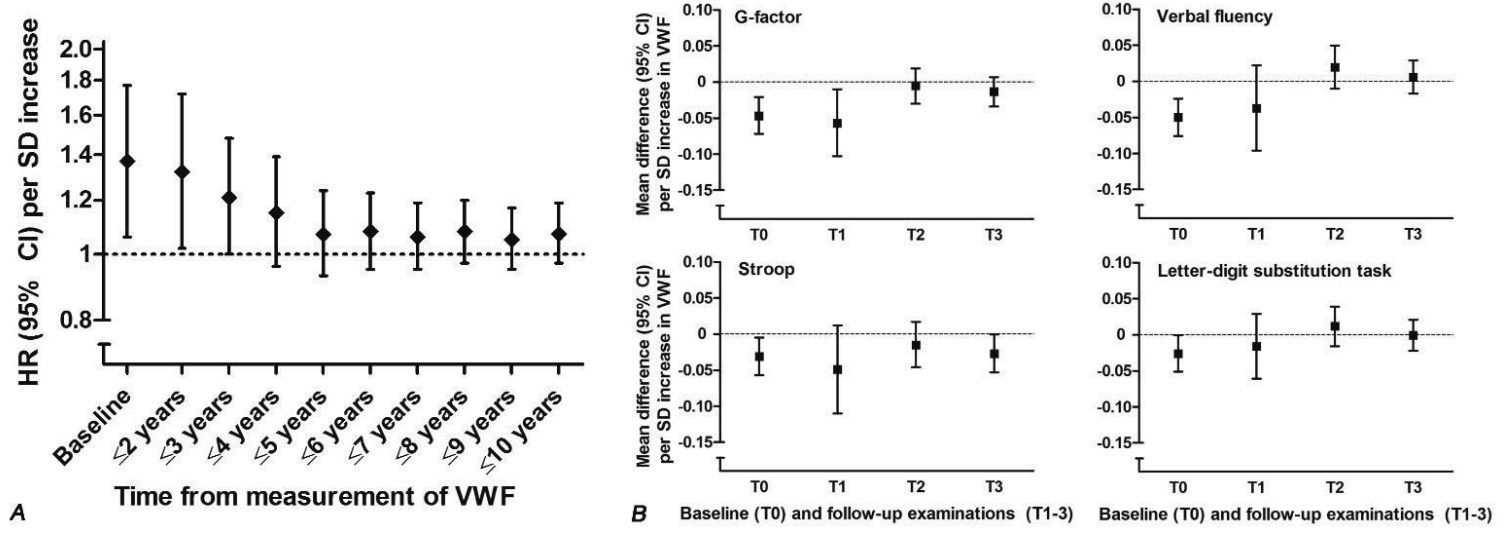


Figure 1. Von Willebrand factor (VWF), change in cognitive performance, and dementia risk. Panel A shows the cross-sectional estimates at baseline (odds ratio from logistic regression), followed by hazard ratios (from Cox regression) for the risk of incident dementia in longitudinal analyses with incremental inclusion of one extra year of follow-up. Results are from the fully adjusted model. In panel B, results reflect the betas per standard deviation increase for baseline VWF and the VWF*follow-up time interaction (expressed per 10 years follow-up) from a fully adjusted linear mixed model including all four examinations, and restricting analyses to the first two or three assessments, respectively. Lower scores reflect worse performance for all tests. Presented cross-sectional estimates from the model including all examinations were robust in the time-restricted models. T0=baseline; T1=first follow-up examination after 4.4 years; T2=second follow-up examination after 10.8 years; T3=third follow-up examination after 15.4 years. HR=hazard ratio; CI=confidence interval; SD=standard deviation.

Low ADAMTS13 activity was associated with an increased risk of dementia (Table 3), with similar effect estimates throughout follow-up. The association was modified by the presence of impaired fasting glucose or diabetes (*P*-interaction=0.003), such that low activity of ADAMTS13 related to higher risk of dementia primarily in non-diabetics, but not in those with (pre-)diabetes (Table 3). This opposite direction of effect was seen for impaired fasting glucose and diabetes, and unaffected by excluding individuals on antidiabetic medication. Risk estimates of ADAMTS13 itself were consistently stronger than those of the ADAMTS13:VWF ratio (data not shown). In contrast to ADAMTS13 there was no interaction between (pre-)diabetes and VWF on dementia risk (*P*-value for interaction=0.99).

ADAMTS13 was associated with more rapid decline in cognitive test performance during 15 years of follow-up (Figure 2), again most profound in individuals without diabetes. The ADAMTS13:VWF ratio was also associated with change in cognitive test performance, with similar effect estimates, except for a somewhat stronger association with the Stroop test. These associations were broadly unaltered after excluding participants who developed dementia during follow-up.

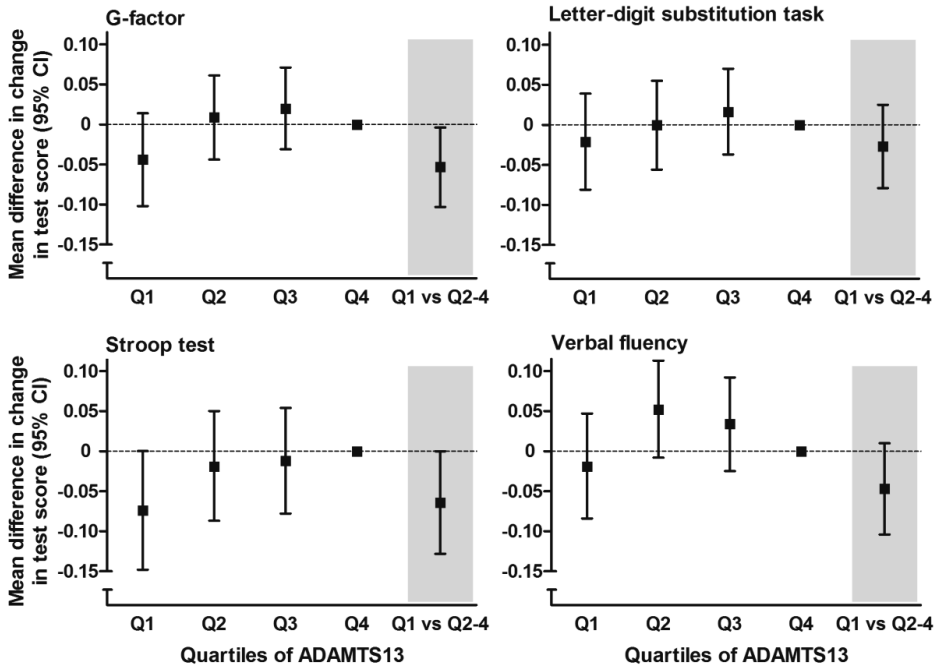


Figure 2. ADAMTS13 activity and change in cognitive test performance. Change in cognitive performance during four consecutive examination rounds, expressed as change per 10 years relative to the highest quartile of ADAMTS13, and comparing low versus normal ADAMTS13 activity. Lower scores reflect worse performance for all tests. Results are from the fully adjusted model.

ADAMTS13 activity	Overall study population		Free of (pre-)diabetes		With (pre-)diabetes	
	n_{dem}/N_{tot}	HR (95% CI)	n_{dem}/N_{tot}	HR (95% CI)	n_{dem}/N_{tot}	HR (95% CI)
Per quartile						
Q1 $_{<80.6\%}$	239/1,492	1.16 (0.94-1.43)	190/1081	1.51 (1.16-1.95)	49/401	0.64 (0.43-0.95)
Q2 $_{80.6\text{-}91.2\%}$	210/1,493	1.00 (0.82-1.23)	158/1088	1.22 (0.94-1.57)	51/389	0.67 (0.46-0.97)
Q3 $_{91.2\text{-}101.9\%}$	191/1,493	0.85 (0.69-1.04)	133/1095	0.90 (0.69-1.17)	58/391	0.77 (0.54-1.10)
Q4 $_{>101.9\%}$	181/1,492	REFERENCE	106/995	REFERENCE	74/482	REFERENCE
Q1 versus Q2-4		1.23 (1.05-1.44)		1.44 (1.20-1.73)		0.81 (0.58-1.13)
Per SD decrease	821/5,970	1.06 (0.98-1.15)	587/4259	1.16 (1.06-1.28)	232/1663	0.90 (0.79-1.03)

Table 3. Baseline ADAMTS13 in relation to the risk of dementia in the overall population, and stratified by (pre-)diabetic status. The model is adjusted for age, sex, study subcohort, smoking, systolic and diastolic blood pressure, antihypertensive medication, serum cholesterol, HDL cholesterol and triglycerides, lipid-lowering medication, body mass index, (pre-)diabetes (if applicable), creatinine, antithrombotic medication, CRP, fibrinogen, and *APOE* genotype. HR=hazard ratio; CI=confidence interval; n_{dem}=number of dementia cases and N_{tot}=number of individuals in group, presented for non-imputed data (missing diabetes status, n=48)

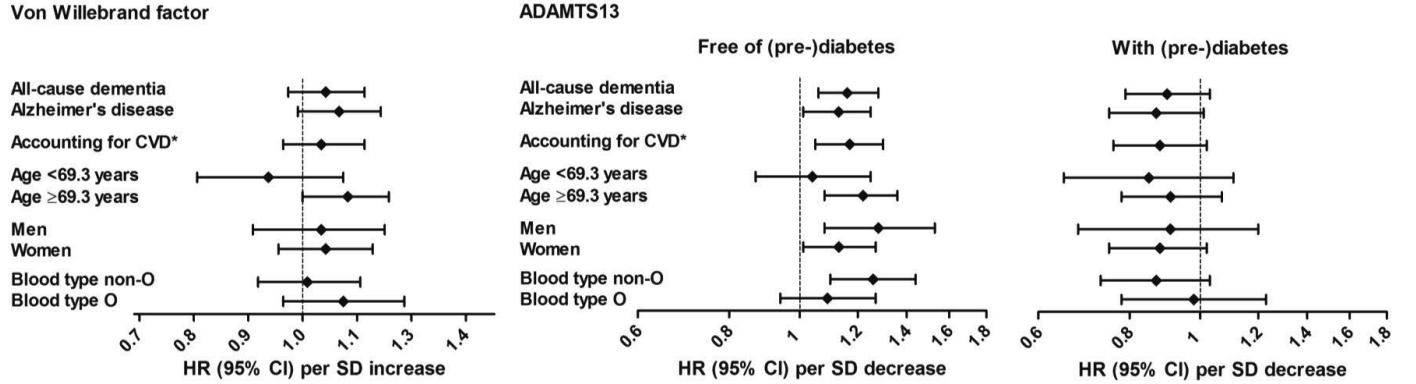


Figure 3. Sensitivity and subgroup analyses for the association of Von Willebrand factor (VWF) and ADAMTS13 with risk of dementia. For age, the population was stratified at the median age of 69.3 years. CVD includes coronary heart disease and stroke. HR=hazard ratio from fully adjusted model; CI=confidence interval; SD=standard deviation.

In further sensitivity analyses, associations of VWF and ADAMTS13 with dementia were similar for Alzheimer's disease only, and unaffected by excluding those with prevalent cardiovascular disease and censoring at time of myocardial infarction or stroke during follow-up (Figure 3). We found no evidence of effect modification by age at blood sampling, sex, or ABO blood type (Figure 3; all *P*-values for interaction≥0.15).

Among a random subset of 1,075 participants with immunoassay biomarker measurements, lower ADAMTS13 activity was significantly associated with higher levels of VEGF, MMP-3, and Tenascin-C, but not ANG-2 and PDGF (Table 4). The univariable association between MMP-3 and ADAMTS13 attenuated after adjustment for age and sex, but differed substantially by concurrent levels of TIMP-1, such that associations were strongest in the presence of high TIMP-1 (0.31 [-1.55;2.17] below the median of TIMP-1 versus -2.93 [-4.96;-1.17] above the median; *P*-value for interaction=0.01). A similar interaction was seen between Tenascin-C and TIMP-1 (-0.68 [-2.16;0.81] below the median, versus -2.09 [-3.57;-0.61] above the median; *P*-value for interaction=0.05).

	Model I β (95% CI)	**Model II** β (95% CI)
ANG-2	0.01 (-1.25; 1.27)	0.72 (-0.62; 2.06)
PDGF	0.64 (-0.41; 1.68)	0.14 (-0.90; 1.19)
VEGF	-1.66 (-3.07; -0.25)*	-1.83 (-3.33; -0.34)*
MMP-3	-4.03 (-5.05; -3.00)*†	-1.37 (-2.64; -0.10)†
Tenascin-C	-1.74 (-2.78; -0.70)*†	-1.42 (-2.46; -0.37)*†
TIMP-1	-1.31 (-2.47; -0.14)*†	1.40 (0.11; 2.69)†

Table 4. ADAMTS13 and selected markers of angiogenesis and extracellular matrix integrity. Values represent change in ADAMTS13 activity per standard deviation increase in the specific marker. Model I is a univariable linear regression; model II also includes age, sex, and all other biomarkers. *statistically significant at 0.05 level after correction for multiple testing; †=the effect estimates for the interaction of TIMP-1 with MMP-3 and Tenascin-C are described in the text. ANG-2=angiopoietin-2; PDGF=platelet-derived growth factor; VEGF=vascular endothelial growth factor; MMP-3=matrix metalloproteinase-3; TIMP-1=tissue inhibitor of metalloproteinases-1.

DISCUSSION

In this large population-based study, we found that higher VWF antigen levels are associated with prevalence and short-term, but not long-term risk of dementia. Low ADAMTS13 activity is associated with dementia risk during prolonged follow-up, with data suggesting an interactive mechanism between ADAMTS13 and diabetes in the development of dementia. We did not observe synergistic effects of VWF and ADAMTS13 activity, which might indeed indicate in part independent underlying mechanisms.

The cross-sectional association between VWF and dementia in our study is in line with a recent meta-analysis, which reported similar (standardised) differences in VWF levels between individuals with all-cause dementia and controls.[10] However, we found associations to rapidly attenuate over the first few years of follow-up, explaining why two prior longitudinal studies did not find a significant association between VWF and risk of dementia after 4 and 17 years of follow-up, respectively.[11,12] The crucial role of time in this association could indicate high variability in VWF levels, either physiologically or induced by disease processes or treatment. Levels of VWF in the bloodstream may increase exponentially during the course of disease due to increasing severity of endothelial injury, and the biological effect of VWF may also vary with physiological changes in advanced stages of disease, such as wall shear stress.[23,24] This physiological variability could be investigated in future studies by incorporation of multiple measurements of VWF over time, which will prove important to determine to which extent prior associations of VWF with (subclinical) disease in fact reflect physiological activity of VWF, or are due to endothelial injury.

VWF has been associated with markers of cerebral small-vessel disease that are known risk factors for dementia,[25,26] including white matter hyperintensities on MRI and microhaemorrhages co-localised with beta-amyloid deposits.[27,28] As high VWF increases the risk of ischaemic stroke,[29] cerebral ischaemia could further link VWF to cognitive decline via (covert) brain infarcts or cortical micro-infarcts. Such effects might be reduced in individuals with blood type O,[30] due to accelerated clearance and thus 25% lower levels of VWF,[31,32] although we did not find differential effects of VWF across blood type in our study. Beyond its direct effects, the function of VWF as a carrier protein for coagulation factor VIII (FVIII), thereby prolonging its half-life tenfold,[33] might in part explain recently reported cognitive impairment with higher FVIII.[30] Finally, in vitro study suggests that inflammatory cytokines increase release and inhibit cleavage of VWF,[34] which might link inflammatory and ischaemic pathways in the pathogenesis of Alzheimer's disease.[14] Future studies linking measurements of VWF to (longitudinal) magnetic resonance neuroimaging may further unravel these potential mechanisms.

In contrast to findings for VWF antigen, low ADAMTS13 activity was associated with cognitive decline and dementia risk throughout the 15-year follow-up in individuals without (pre-)diabetes. In line with reports of myocardial infarction and ischaemic stroke,[5,8] we observed increased risks only in the lower range of ADAMTS13, supporting a threshold effect in ADAMTS13 activity.[2] Nevertheless, the lower range of activity in the community is generally sufficient to maintain the equilibrium of VWF multimer formation and degradation.[35,36] Along with the effect estimates for ADAMTS13 generally exceeding those of

the ADAMTS13:VWF ratio, this renders it unlikely that proteolytic effects of ADAMTS13 on VWF alone are accountable for the association of ADAMTS13 with dementia. Yet, most studies about ADAMTS13 have focused on its relationship with VWF or role in thrombotic thrombocytopenic purpura, and limited data are available to corroborate other pathways. Preliminary evidence suggests a role of ADAMTS13 in (downregulation of) inflammation,[13,37] regulation of angiogenesis,[13] and degradation of extracellular matrix,[13] which have also been described in dementia.[14-16] In mice, deficiency of ADAMTS13 enhances inflammation and plaque formation,[38,39] aggravates consequences of cerebral ischaemia,[40-42] and appears to regulate blood-brain barrier permeability,[43] possibly by controlling vascular remodeling via VEGF, ANG-2, and galectin-3 related pathways.[42,43] While these processes in mice often appear dependent on VWF or are observed in ADAMTS-/- mice, the levels required may be limited, and thus generally abundant in the general population. In exploratory analyses, we found associations of ADAMTS13 activity with levels of VEGF, MMP-3, Tenascin-C, and TIMP-1, which might indeed indicate involvement in vascular remodelling, and in any case encourage further study of ADAMTS13 in relation to vascular (brain) disease and neurodegeneration.

Our findings suggest that diabetes pathophysiology, rather than antidiabetic medication, modifies the association between ADAMTS13 and dementia risk. These analyses were prompted by our recent study in which we found increased risks of diabetes with higher ADAMTS13 activity.[17] Although the mechanisms underlying these observations are unknown, it is conceivable that ADAMTS13 has other, yet unidentified proteolytic activity, or competes/ interacts with glucose or currently unknown protein(s) to contribute to cognitive decline. One would expect that the pathological mechanism underlying this interaction shows similarly in the association of ADAMTS13 with related disease outcomes. A previous report of the Rotterdam Study has described an increased risk of ischaemic stroke with low ADAMTS13 activity,[5] but the link between ADAMTS13 and diabetes had not yet emerged at the time. Exploring these data further in a post-hoc analysis, we now observed patterns in the association between ADAMTS13 and risk of ischaemic stroke, similar to those with dementia in the current study (HR [95% CI] per SD decrease in ADAMTS13 for risk of ischaemic stroke in those free of (pre-)diabetes: 1.19 [1.04-1.36], versus in those with (pre-)diabetes: 0.94 [0.79-1.11]). This points towards a vascular disease related interactive mechanism, in which ADAMTS13 has a common role across diseases outcomes. While we encourage attempts for replication of our findings in other populations, we believe that current insight warrants serum glucose and diabetes history to be taken into account in future study of ADAMTS13.

Although we believe our results are reliable, there are several limitations. First, despite rigorous adjustment for known determinants of VWF and ADAMTS13, residual confounding may still exist, in particular with respect to other factors involved in hemostasis, diabetes, or possibly angiogenesis and extracellular matrix stability. Second, although follow-up for dementia was near-complete, attrition for repeated detailed cognitive assessment was substantial. Third, the association between ADAMTS13 and diabetes was first described in the same cohort as drawn from in the present analyses, and (large-scale) replication is warranted. Fourth, the Rotterdam Study population is predominantly of Caucasian descent, and levels and effects of ADAMTS13 might differ across ethnicities.

In conclusion, higher VWF and low ADAMTS13 activity are associated with accelerated cognitive decline and increased risk of dementia. However, associations with VWF are restricted to short-term risks, and do not display synergistic effects with ADAMTS13 on dementia risk. The impact of diabetes on the effect of ADAMTS13 on dementia (as well as ischaemic stroke), further emphasises the need to unravel the biological function of ADAMTS13.

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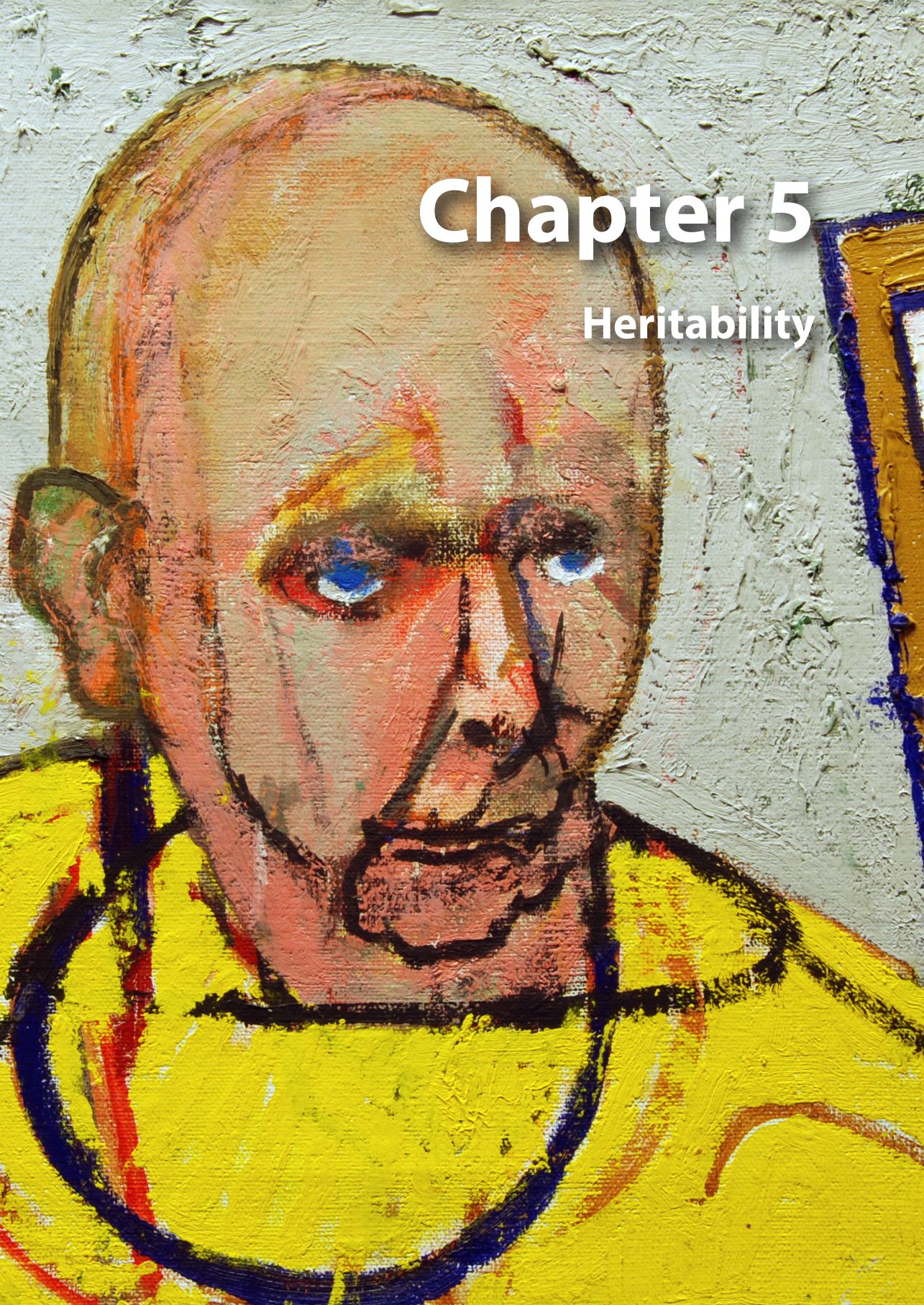
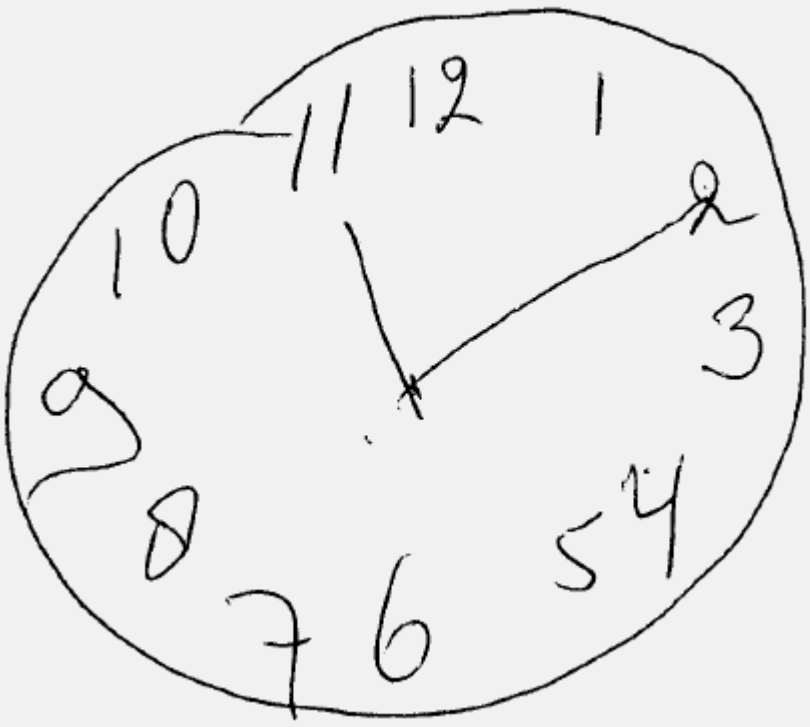


Chapter 5

Heritability

Chapter 5.1

APOE and mortality



ABSTRACT

Apolipoprotein E is a glycoprotein best known as a mediator and regulator of lipid transport and uptake. The *APOE*-ε4 allele has long been associated with increased risks of Alzheimer's disease and mortality, but the effect of the less prevalent *APOE*-ε2 allele on diseases in the elderly and survival remains elusive. We aggregated data of 38,537 individuals of European ancestry (mean age 66 years, 56% women) from six population-based cohort studies (Framingham Heart Study, Rotterdam Study, AGES-Reykjavik Study, Long Life Family Study, Cardiovascular Health Study, Health-ABC Study) to determine the association of *APOE*, and in particular *APOE*-ε2, with survival in the general population. During a mean follow-up of 11.7 years, 17,021 individuals died. Compared with homozygous *APOE*-ε3 carriers, *APOE*-ε2 carriers were at lower risk of death (hazard ratio [95% confidence interval]: 0.94 [0.90-0.99], P=1.1*10^{-2}), whereas *APOE*-ε4 carriers were at increased risk of death (HR 1.17 [1.12-1.21], P=2.8*10^{-16}). *APOE* was associated with mortality risk in a dose-dependent manner, with risk estimates lowest for homozygous *APOE*-ε2 (HR 0.89 [0.74-1.08]), and highest for homozygous *APOE*-ε4 (HR 1.52 [1.37-1.70]). Results were broadly similar across cohorts, and did not differ by age or sex. *APOE* genotype was associated with baseline lipid fractions (e.g. mean difference [95%CI] in LDL [mg/dL] for ε2 versus ε33: -17.1 [-18.1;-16.0], and ε4 versus ε33: +5.7 [4.8;6.5]), but the association between *APOE* and mortality was unaltered after adjustment for baseline LDL or cardiovascular disease. In conclusion, compared with *APOE*-ε3, *APOE*-ε2 is associated with prolonged survival, whereas mortality risk is increased for *APOE*-ε4 carriers. Further collaborative efforts are needed to unravel the role of *APOE* and in particular *APOE*-ε2 in health and disease.

INTRODUCTION

Apolipoprotein E is a glycoprotein best known as a mediator and regulator of lipid transport and uptake, but also has several additional physiological and pathological roles.[1] The *APOE* gene, on chromosome 19, contains four exons and codes for a 317 amino acid polypeptide that gives rise to a 299 amino acid long mature protein (34kD).[1] There are three circulating *APOE* isoforms designated *APOE*-ε4, -ε3, and -ε2, with corresponding allele frequencies of approximately 14%, 78%, and 8%, respectively.[2] Within the central nervous system, apolipoprotein E is produced mainly by astrocytes, while in peripheral tissue, it is expressed primarily in the liver and kidneys in addition to spleen, adrenals, and fatty tissue.[3,4]

Various studies, dating back as far as 25 years ago, have shown that allelic variation at the *APOE* locus impacts survival, and alters risk of hyperlipidaemia, atherosclerosis, cardiovascular disease, and in particular dementia.[5-7] Initial attention was largely focused on the *APOE*-ε4 allele, which is associated with an adverse impact on these risk factors and outcomes, including shortened survival compared with the more common ε3 allele. More recent data suggest that the *APOE*-ε2 allele might prolong survival,[8-11] but other studies do not support such an association,[12,13] and have even implicated the ε2 allele as a detrimental factor in cerebral small-vessel disease,[14] dysbetalipoproteinemia,[15] and aggressiveness of certain cancer.[16] A better understanding of the benefits and risks associated with *APOE*-ε2 carrier status, above and beyond the absence of the ε4 allele, could lead to novel preventive and treatment options for a wide variety of conditions to promote healthy aging and longevity. Yet, studies of the ε2 allele have been hampered by its low allele frequency, which results in only 1% of the population being homozygous ε2 carriers. Larger studies are therefore warranted, requiring collaborative efforts to design well-powered studies to address these questions.

We aggregated data from six large cohorts, and aimed to determine the impact of the *APOE*-ε2 allele on survival in the general population. In addition, we studied potential vascular or lipid-mediated mechanisms that might account for this association.

METHODS

Study population

This study population consisted of participants of European ancestry from six population-based cohort studies: the Framingham Heart Study (FHS), the AGES-Reykjavik Study (AGES), the Rotterdam Study (RS), the Cardiovascular Health Study (CHS), the Long-Life Family

Studies (LLFS), and the Health, Aging, and Body Composition study (HABC). Details of the design and characteristics of participating studies have been described previously,[17-23] and are summarised below. All studies were approved by the relevant institutional review boards, and written informed consent was obtained from all participants.

The **Framingham Heart Study (FHS)** was initiated to study determinants of cardiovascular disease. The original cohort was recruited in 1948 and the offspring of the Original cohort participants and offspring spouses were enrolled in 1971.[17,18] DNA was obtained for genetic studies in the 1990s from surviving Original cohort and Offspring participants. Year 1990 is considered the baseline exam for these analyses. All participants remain under continuous surveillance and deaths that occurred through 31st December 2013 were included in the present analyses. The **Age, Gene/Environment Susceptibility -Reykjavik Study (AGES)** was initiated to examine potential genetic susceptibility and gene/environment interaction.[19] Between 2002 and 2006, baseline exams were conducted in survivors from the Reykjavik Study. Follow-up information was complete till 31st December 2015 via linkage to electronic medical records and vital status registry. Between 1990 and 1993 all inhabitants of the Ommoord district in Rotterdam, The Netherlands, aged ≥55 years were invited to participate in the **Rotterdam study (RS).**[20] The cohort was subsequently expanded with inhabitants who moved into the area or reached eligible age in 2000 (≥55 years) and 2005 (≥45 years). Participants were interviewed at home and examined at the study centre every four years. Continuous surveillance of general practitioners' records, hospital records, and death certificates was used for identification of disease events and deaths through 1st January 2015. The **Cardiovascular Health Study (CHS)** is a prospective population-based cohort study of cardiovascular disease and mortality in >65 years old Medicare-eligible adults living in four United States communities.[21] Recruitment of the initial cohort was completed in 1990 and 3,267 participants fulfilled the inclusion criteria of this study and had genotyping information available. Only European or European Americans, who consented to the use of their genetic data, were included in the present analyses. Major incident health events and deaths were identified through several methods, including 1) questionnaires completed by participants at each semi-annual contact during follow-up; 2) reports by family members; and 3) periodic searches of the Medicare Utilization database, the National Death Index, and local newspaper obituaries. Follow-up for the analysis here was complete till 30th June 2014. The **Long-Life Family Study (LLFS)** enrolled families enriched for longevity via four field centres (Boston, New York, and Pittsburgh in the USA, and Denmark) between 2006 and 2009.[22] The recruitment protocol used the Family Longevity Selection Score (FLoSS) to identify family enriched of exceptional longevity, and enrolled 583 families with a FLoSS $\geq$7 consisting of 1493 probands, their siblings and 192 spouses in the older generation, and 2437 offspring and 809 of their spouses. Information collected on onsets of diseases was assessed

retrospectively at baseline from self-reports and prospectively during in-person visit (home or clinic), self-administration, or telephone interview through 2015. Death was assessed annually by interview of proxies or from nationwide survival and health register (Denmark) through 2015. The **Health, Aging, and Body Composition study (HABC)** is a prospective cohort study of 3,075 community-dwelling black and white men and women living in Memphis, TN, or Pittsburgh, PA, and aged 70–79 years at recruitment in 1996-1997.[23] Participants were a random sample of Medicare-eligible elders within designated zip code areas. The present analyses include participants of self-designated European ancestry, who consented to the use of their genetic data. After baseline examination, participants were re-examined annually, and surveilled through phone contacts every 6 months to identify major health events and document functional status between clinic visits. In addition, the study collects and abstracts medical records of all hospitalizations (≥24 hours) and adjudicates the occurrence of targeted health events including all deaths. Dates and causes of death were obtained from death certificates until September 2014. A Health ABC Committee representing all the study units adjudicated causes of death based on the review of medical records, proxy information and autopsy report (when performed).

***APOE* genotyping**

APOE genotype was determined directly (i.e. not using genetic imputations) in all cohorts. Methods that were used include polymerase chain reaction on coded DNA samples (RS original cohort, FHS 1st and 2nd generation, CHS, AGES, Health ABC) and bi-allelic TaqMan assays (rs7412 and rs429358) (RS expansion cohorts, FHS 3rd generation, LLFS).

Other measurements

Fasting serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured at baseline. Low-density lipoprotein (LDL) was computed from total and HDL cholesterol and triglycerides, using Friedewald's formula.[24] Use of lipid-lowering medication was assessed at baseline by interview. Prevalence of heart disease (including myocardial infarction, angina (or coronary revascularisation for the Rotterdam sample), heart failure, and cerebrovascular disease (including stroke and transient ischemic attack) was ascertained by interview, and confirmed by medical records and/or electrocardiography.

Analysis

For each cohort separately, we used Cox proportional hazard models (with robust variance for family cohorts) to determine the association between *APOE* genotype and death, while adjusting for age, sex, and centre of ascertainment (if applicable). Analyses included a comparison of each of the *APOE* genotypes to ε3:ε3, as well as a comparison of ε2 carriers versus ε3 homozygotes, and ε4 carriers versus ε3 homozygotes. For appropriateness of

comparison, heterozygote ε2:ε4 carriers were excluded from the latter analyses. We explored interaction of *APOE* with age and sex, by testing for multiplicative interaction.

Next, triglyceride levels were log-transformed to obtain a roughly normal distribution of data. We then determined per cohort differences in total cholesterol, HDL cholesterol, triglycerides, and LDL across *APOE* genotypes, using linear regression (mixed effects model for family cohorts), adjusting for age, sex, ascertainment center, and use of lipid-lowering medication. In two cohorts (FHS and RS), we assessed the additional variance explained by *APOE* genotype. We then repeated the survival analyses with additional adjustment for measured lipid fractions, and prevalent cardiovascular disease.

We used inverse variance weighted fixed and random effects models to pool hazard ratios and mean differences from separate cohorts. We formally assessed for heterogeneity between studies, determining the share of variation across studies that was due to heterogeneity rather than chance (Higgins' I^2 statistic).[25] In case of substantial heterogeneity (>40%), we report results of random rather than fixed effects meta-analysis.

Analyses were done using SPSS Statistics version 23.0 (IBM Corp, Armonk, NY, USA) or R statistical software version 3.1.1 ('survival' and 'meta' packages). Alpha level was set at 0.05.

RESULTS

A total of 38,537 participants were included from the 6 cohort studies. Baseline characteristics of the entire sample as well as per cohort are presented in Table 1. The allele frequency of the *APOE*-ε2, ε3, and ε4 alleles was 7.9%, 78.6%, and 13.5%, respectively. Observations lay within Hardy-Weinberg equilibrium.

During 429,708 person years of follow-up (mean 11.7 years), 17,021 participants died. Carrying one or two copies of the ε2 allele was significantly associated with reduced mortality risk (hazard ratio (HR) [95% confidence interval]: 0.94 [0.90-0.99], $P=1.1*10^{-2}$; Figure 1), whereas *APOE*-ε4 carriers were at increased risk of death (HR 1.17 [1.12-1.21], $P=2.8*10^{-16}$; Figure 1). *APOE* genotype was associated with survival in a dose-dependent manner, such that mortality risk was lowest for homozygous ε2 carriers, and highest for homozygous ε4 carriers (Figure 2, along with Table 2 for the results per study). Risk for individuals with the ε2:ε4 genotype was most comparable to their ε3:ε4 rather than their ε2:ε3 counterparts. The associations were broadly similar across cohorts (Figure 1), and there was no evidence of interaction with age at study entry or sex (data not shown).

Characteristics	Overall sample*	AGES	CHS	FHS	HABC	LLFS	RS
Sample size	38537	5740	4397	9304	1712	4630	12754
Age, years	65.5	77.0 (±5.9)	72.8 (±5.6)	51.2 (±15.2)	73.8 (±2.9)	70.3 (±15.8)	65.4 (±10.0)
Male sex	17091 (44.4%)	2429 (42.3%)	1904 (43.3%)	4242 (45.6%)	939 (52.3%)	2211 (47.5%)	5385 (42.2%)
Current smoking	5639 (14.6%)	679 (12.2%)	489 (11.1%)	1424 (16.3%)	111 (6.5%)	313 (7.3%)	2623 (21.2%)
Hypertension	19628 (50.9%)	4618 (81.1%)	2455 (55.9%)	2711 (31.0%)	671 (39.1%)	2252 (48.4%)	6921 (55.1%)
Body-mass index	26.9	27.0 (±4.5)	26.3 (±4.4)	27.1 (±5.2)	26.5 (±4.1)	27.1 (±4.9)	26.9 (±4.1)
Diabetes	3268 (8.5%)	740 (12.9%)	629 (14.4%)	469 (5.4%)	194 (11.3%)	180 (4.3%)	1056 (8.6%)
Total cholesterol, mg/dL	216.3	217.5 (±44.8)	211.8 (±39.2)	199.0 (±37.3)	201.5 (±37.6)	199.7 (±42.2)	238.0 (±47.9)
HDL cholesterol, mg/dL	54.7	61.3 (±17.3)	53.7 (±15.8)	51.7 (±15.8)	51.9 (±16.3)	58.8 (±17.3)	53.1 (±15.2)
Triglycerides, mg/dL	129.3	108.6 (±60.7)	143.3 (±78.1)	130.0 (±112.1)	152.6 (±87.6)	113.4 (±72.1)	135.9 (±75.1)
Triglycerides (transformed)†	4.73	4.57 (±0.46)	4.86 (±0.43)	4.69 (±0.56)	4.90 (±0.48)	4.59 (±0.51)	4.80 (±0.45)
LDL cholesterol, mg/dL	129.3	134.8 (±40.1)	130.3 (±35.6)	118.6 (±33.0)	119.8 (±33.2)	118.6 (±35.8)	139.4 (±35.9)
Lipid lowering medication	6362 (16.5%)	1249 (21.8%)	230 (5.2%)	606 (6.9%)	880 (51.4%)	2169 (43.4%)	1228 (9.7%)
APOE genotype							
ε3/ε3	23813 (61.9%)	3558 (62.0%)	2747 (62.5%)	6015 (64.6%)	1082 (63.2%)	3031 (65.1%)	7434 (58.3%)
ε2/ε2	239 (0.6%)	30 (0.5%)	28 (0.6%)	47 (0.5%)	13 (0.8%)	33 (0.7%)	89 (0.7%)
ε2/ε3	4721 (12.3%)	518 (9.0%)	560 (12.7%)	1140 (12.3%)	212 (12.4%)	695 (14.9%)	1605 (12.6%)
ε2/ε4	873 (2.3%)	115 (2.0%)	104 (2.4%)	183 (2.0%)	28 (1.6%)	87 (1.9%)	357 (2.8%)
ε3/ε4	8129 (21.1%)	1397 (24.3%)	904 (20.6%)	1764 (19.0%)	353 (20.6%)	762 (16.4%)	2965 (23.2%)
ε4/ε4	706 (1.8%)	122 (2.1%)	54 (1.2%)	155 (1.7%)	24 (1.4%)	48 (1.0%)	304 (2.4%)

Table 1. Baseline characteristics. Values are depicted as means ±SD and absolute numbers (%); *derived from summary statistics; †natural log transformed.

		AGES	CHS	FHS	HABC	LLFS	RS
	N_{mort}/N_{total}	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
***APOE* genotype**							
ε2/ε2	107/239	0.74 (0.41-1.35)	0.96 (0.65-1.43)	1.20 (0.70-2.08)	0.67 (0.30-1.50)	1.13 (0.53-2.42)	0.82 (0.61-1.10)
ε2/ε3	2027/4728	0.98 (0.86-1.12)	0.95 (0.87-1.05)	0.89 (0.76-1.03)	0.96 (0.79-1.17)	0.94 (0.78-1.13)	0.94 (0.87-1.02)
ε3/ε3	10405/23847	Reference	Reference	Reference	Reference	Reference	Reference
ε2/ε4	410/873	1.20 (0.94-1.53)	0.98 (0.79-1.22)	1.25 (0.86-1.82)	1.44 (0.90-2.29)	1.02 (0.61-1.70)	1.15 (0.99-1.34)
ε3/ε4	3747/8143	1.19 (1.09-1.29)	1.14 (1.06-1.24)	1.15 (1.03-1.30)	1.16 (0.99-1.36)	1.17 (0.96-1.43)	1.12 (1.05-1.19)
ε4/ε4	325/707	1.54 (1.21-1.95)	1.59 (1.21-2.10)	1.99 (1.49-2.64)	1.25 (0.76-2.05)	1.89 (0.86-4.16)	1.38 (1.17-1.63)

Table 2. *APOE* and mortality per *APOE* genotype per study. N_{mort}=number of individuals who died; N_{total}=total sample size; HR=hazard ratio; CI=confidence interval.

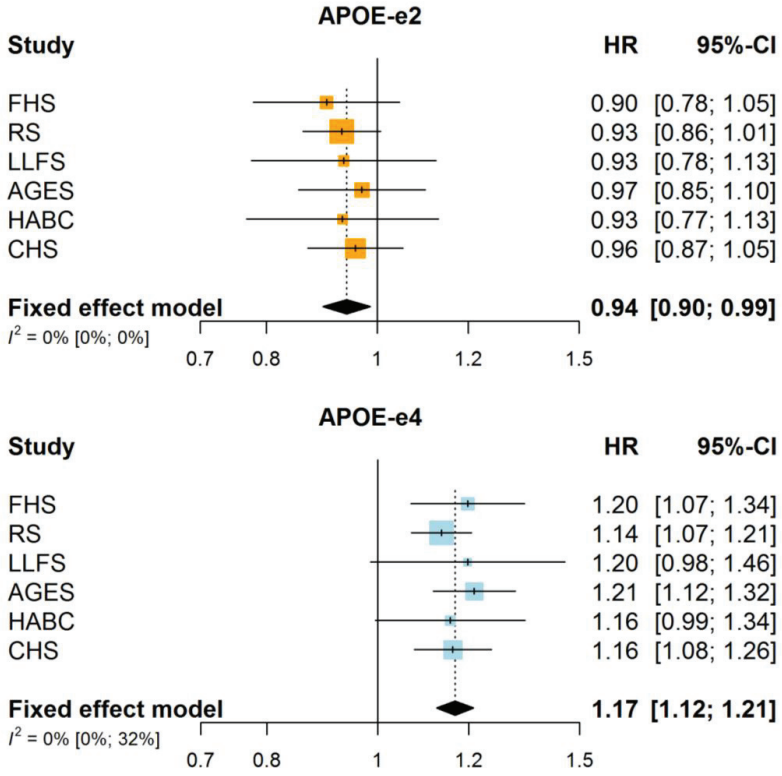


Figure 1. *APOE* and mortality. Association of *APOE*-ε2 and *APOE*-ε4 carrier status with mortality per cohort, and meta-analysis. HR=hazard ratio; CI=confidence interval.

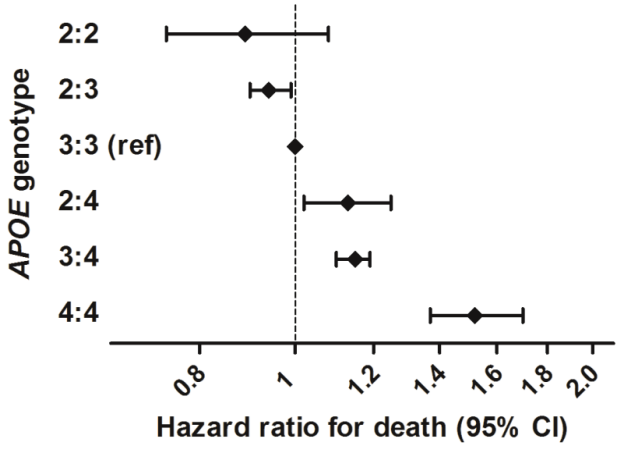


Figure 2. *APOE* and mortality. Meta-analysed effect estimates of the associations between *APOE* genotypes and mortality. CI=confidence interval.

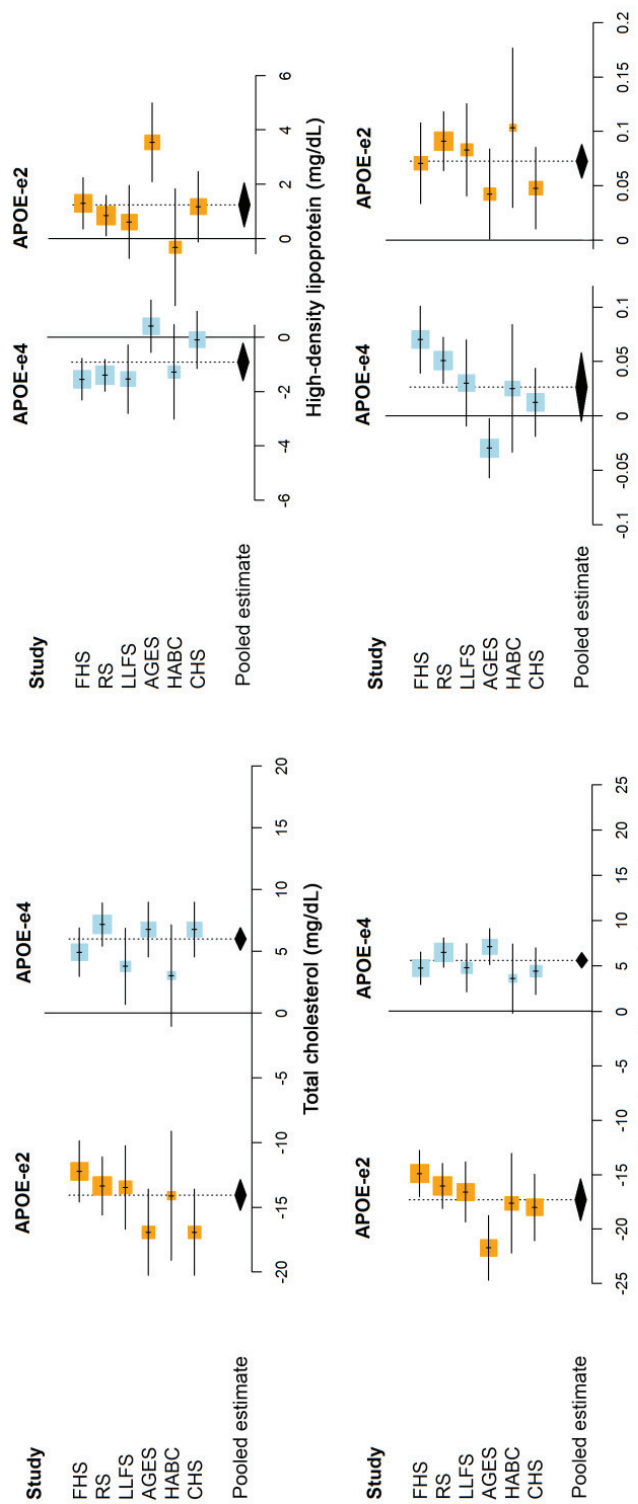


Figure 3. *APOE* and lipid fractions. Mean differences in lipid fractions of *APOE*-ε2 (orange) and *APOE*-ε4 (light blue) compared to homozygous ε3 carriers, per cohort and meta-analysis

	N_{total}	AGES Mean differences (95% CI)	CHS Mean differences (95% CI)	FHS Mean differences (95% CI)	HABC Mean differences (95% CI)	LLFS Mean differences (95% CI)	RS Mean differences (95% CI)
Total cholesterol							
ε2/ε2	235	-32.1 (-45.4;-18.8)	-45.0 (-58.6;-31.4)	-30.2 (-40.9;-19.5)	-7.9 (-26.8;10.9)	-22.4 (-36.0;-8.8)	-11.2 (-20.0;-2.3)
ε2/ε3	4628	-16.1 (-19.6;-12.7)	-13.0 (-16.3;-9.7)	-11.8 (-14.2;-9.4)	-14.5 (-19.6;-9.4)	-13.3 (-16.6;-10.1)	-13.5 (-15.8;-11.2)
ε3/ε3	23302	REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE
ε2/ε4	858	-4.3 (-11.2;2.6)	-13.0 (-20.2;-5.8)	-4.4 (-9.8;1.1)	-5.8 (-18.7;7.2)	-6.7 (-15.1;1.6)	-5.7 (-10.3;-1.2)
ε3/ε4	7939	6.1 (3.8;8.4)	4.1 (1.4;6.9)	4.6 (2.6;6.6)	3.1 (-1.2;7.3)	3.7 (0.6-6.9)	6.9 (5.1;8.7)
ε4/ε4	689	14.6 (7.9;21.3)	15.8 (5.9;25.7)	7.8 (2.0;13.7)	3.4 (-10.6;17.4)	4.7 (-6.5;15.8)	9.5 (4.6;14.4)
Low-density lipoprotein							
ε2/ε2	193	-43.2 (-55.2;-31.3)	-52.0 (-65.3;-38.7)	-39.0 (-49.2;-28.9)	-18.2 (-37.0;0.5)	-32.6 (-44.3;-20.9)	-29.0 (-38.0;-20.1)
ε2/ε3	4132	-20.6 (-23.6;-17.5)	-16.0 (-19.1;-12.8)	-14.2 (-16.4;-12.1)	-17.6 (-22.3;-12.9)	-16.1 (-19.0;-13.3)	-16.0 (-18.1;-13.8)
ε3/ε3		REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE
ε2/ε4	744	-6.9 (-13.1;-0.8)	-13.0 (-19.8;-6.2)	-7.6 (-12.6;-2.6)	-10.1 (-22.3;2.1)	-13.5 (-20.7;-6.3)	-7.7 (-12.0;-3.5)
ε3/ε4	7029	6.5 (4.4;8.6)	4.0 (1.4;6.7)	4.4 (2.5;6.2)	3.3 (-0.6;7.2)	4.7 (2.0;7.4)	6.5 (4.8;8.2)
ε4/ε4	589	13.4 (7.4;19.4)	10.4 (1.0;19.7)	7.6 (2.2;13.0)	7.9 (-4.9;20.8)	5.1 (-4.5;14.7)	9.2 (4.6;13.8)
High-density lipoprotein							
ε2/ε2	235	5.6 (-0.3;11.4)	0.4 (-5.0;5.8)	5.6 (1.4;9.7)	0.1 (-8.3;8.4)	0.6 (-5.0;6.2)	0.5 (-2.4;3.5)
ε2/ε3	4619	3.4 (1.9;4.9)	1.2 (-0.1;2.5)	1.2 (0.2;2.1)	-0.4 (-2.6;1.9)	0.4 (-0.9;1.8)	0.9 (0.1;1.6)
ε3/ε3	23277	REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE
ε2/ε4	856	1.4 (-1.6;4.4)	-2.1 (-4.9;0.7)	-0.4 (-2.5;1.7)	-2.5 (-8.2;3.2)	0.9 (-2.6;4.3)	0.1 (-1.4;1.6)
ε3/ε4	7921	0.4 (-0.6;1.4)	-0.3 (-1.4;0.8)	-1.4 (-2.2;-0.6)	-1.2 (-3.0;0.6)	-1.6 (-2.9;-0.3)	-1.3 (-2.0;-0.7)
ε4/ε4	688	0.1 (-2.8;3.0)	3.8 (-0.2;7.7)	-3.2 (-5.5;-0.9)	-2.4 (-8.5;3.8)	0.4 (-4.2;4.9)	-1.9 (-3.5;-0.3)
Triglycerides*							
ε2/ε2	185	0.22 (0.06;0.38)	0.06 (-0.10;0.22)	0.13 (-0.04;0.30)	0.23 (-0.06;0.52)	0.25 (0.07;0.43)	0.25 (0.13;0.37)
ε2/ε3	3930	0.03 (-0.01;0.07)	0.05 (0.01;0.09)	0.07 (0.03;0.10)	0.10 (0.02;0.17)	0.08 (0.03;0.12)	0.08 (0.05;0.11)
ε3/ε3	20759	REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE	REFERENCE
ε2/ε4	722	0.06 (-0.02;0.15)	0.09 (0.004;.17)	0.08 (-0.01;0.17)	0.15 (-0.05;0.34)	0.11 (0.003;0.22)	0.12 (0.06;0.18)
ε3/ε4	6700	-0.04 (-0.06;-0.01)	0.01 (-0.02;0.04)	0.06 (0.03;0.10)	0.03 (-0.03;0.09)	0.03 (-0.01;0.08)	0.05 (0.03;0.07)
ε4/ε4	561	0.03 (-0.05;0.11)	0.02 (-0.09;0.14)	0.12 (0.03;0.22)	-0.01 (-0.22;0.19)	-0.03 (-0.18;0.11)	0.07 (0.01;0.13)

Table 3. Lipid fractions by separate APOE genotypes per study. *natural log-transformed; CI=confidence interval.

APOE genotype was associated with all measured lipid fractions, generally in a dose-dependent manner (Figures 3 and 4; and for the full results per cohort Table 3). Compared with homozygous ε3 carriers, levels of total, LDL, and HDL cholesterol were lower in ε2 and higher in ε4 carriers, whereas both ε2 and ε4 carriers had higher levels of triglycerides. The ε2 allele was associated with greater absolute changes in lipid levels than the ε4 allele. Accordingly, levels in those with ε2:ε4 genotype were generally more consistent with ε2 than with ε4 carriership (Figure 4). Comparing ε2 and ε4 carriers with ε3 homozygotes, standardised mean differences in LDL cholesterol exceeded differences in triglycerides and HDL cholesterol (Table 4). *APOE* genotype explained 1.6-3.2% of variance in total cholesterol, 3.9-5.5% for LDL cholesterol, 0.3-0.4% for HDL cholesterol, and 0.8-0.9% for triglycerides.

Effect estimates of *APOE* carrier status for mortality risk were not attenuated by adjustment for LDL (pooled estimates [95%CI] for ε2 carriers: 0.91 [0.86-0.96], and for ε4 carriers: HR 1.19 [1.14-1.24]). Similarly, adjustment for prevalent cardiovascular disease did not materially change risk estimates of mortality (pooled estimates [95%CI] for ε2 carriers: 0.95 [0.90-1.00], and for ε4 carriers: HR 1.16 [1.12-1.21]).

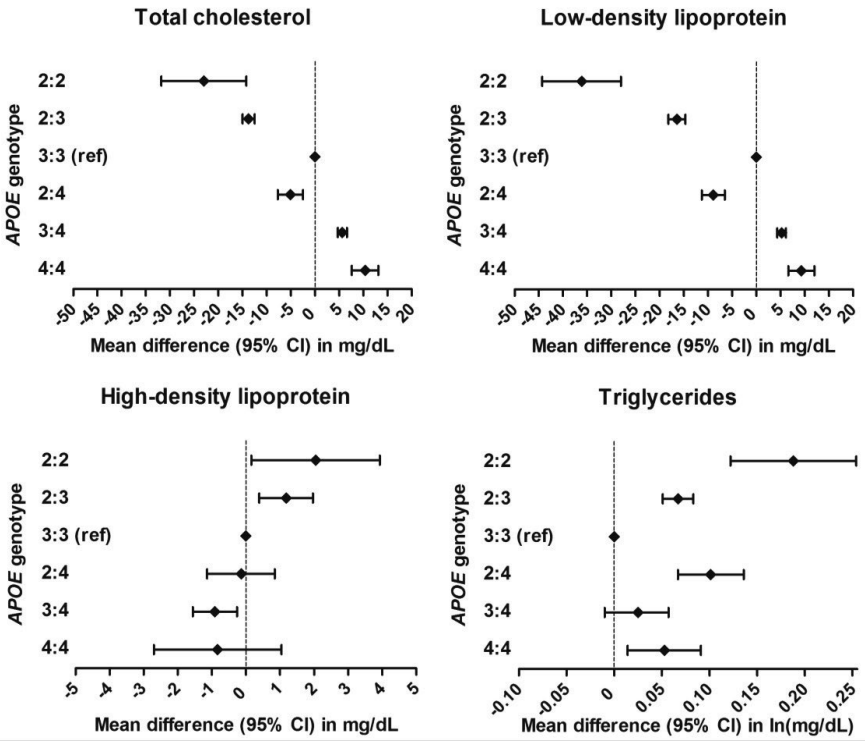


Figure 4. *APOE* and lipid fractions. Meta-analysed effect estimates of the associations between separate *APOE* genotypes and lipid fractions

	Standardised mean difference (95% CI)
Total cholesterol	
ε2 vs ε3/ε3	-0.32 (-0.35;-0.29)
ε4 vs ε3/ε3	0.13 (0.11;0.16)
Low-density lipoprotein	
ε2 vs ε3/ε3	-0.48 (-0.51;-0.45)
ε4 vs ε3/ε3	0.16 (0.13;0.18)
High-density lipoprotein	
ε2 vs ε3/ε3	0.07 (0.05;0.10)
ε4 vs ε3/ε3	-0.06 (-0.09;-0.04)
Triglycerides	
ε2 vs ε3/ε3	0.15 (0.12;0.18)
ε4 vs ε3/ε3	0.06 (0.04;0.09)

Table 4. Standardised differences in lipid fractions by *APOE* genotype. Meta-analysis of standardised effect estimates for the association between different *APOE* genotypes and lipid fractions. CI=confidence interval.

DISCUSSION

E2-CHARGE is the largest collaboration of cohort studies to date to determine the impact of *APOE* and, in particular, the *APOE*-ε2 allele, and aggregates data of 38,537 individuals from 6 population-based cohort studies. In this first analysis of the data, we found that the *APOE*-ε2 allele is associated with prolonged survival, whereas *APOE*-ε4 is associated with increased mortality. Adjustment for the prevalence of cardiovascular disease or measured lipid fractions had trivial effects on these estimates.

Since the first implication of *APOE* in longevity,[26] several genome-wide association and candidate gene studies have aimed to confirm a role of *APOE* in survival. While most of these studies indeed show that *APOE* allele frequencies shift with age,[27] or confirm that *APOE* is associated with longevity as study endpoint, they did not reach genome wide significance,[28-30] or were unable to distinguish effects of variation at the *APOE* locus from those at the *TOMM40* or *APOC1* loci.[31,32] Several cohort studies have examined the association of *APOE* genotype with survival and longevity, but with contrasting findings. Two Scandinavian studies reported hazardous effects of the ε4 allele,[8,12] while one of the two studies also found a protective effect of the ε2 allele.[8] These findings are supported by a lower prevalence of ε4 and a higher prevalence of ε2 in offspring from long-lived families compared to spouse controls,[9] as well as in the very elderly compared to middle-aged populations.[10,11] Nevertheless, neither ε2 nor ε4 were prospectively associated with survival in a very elderly U.S. population, the 90+ Study.[13] We found dose-dependent associations of *APOE* genotype with survival, which were consistent across participating cohorts. Our pooled

effect estimates suggest prior studies were likely underpowered to detect these differences, in particular for the ε2 allele. Survival and selection bias at older ages, whereby ε4 carriers die prior to entry or are less likely to be enrolled due to poor health, may have led to underestimation of hazardous effects of the ε4 allele in others.

Mounting evidence suggests pleiotropic effects of *APOE* on various organ systems. In addition to the well-established link with dementia, more recent clinical and population studies have linked *APOE* gene variation to atherosclerosis,[33] cerebral amyloid angiopathy,[34] stroke,[33] lung disease,[35] multiple sclerosis,[36] and neoplasia.[37] Preclinical studies have put forward intriguing hypotheses of molecular pathways, relating to cerebrovascular function,[38,39] neuronal growth regulation,[40] inflammation,[3] functions as a protein chaperone,[41] type III hyperlipoproteinemia,[15,42] prostate tumor aggressiveness,[16] and epigenetic regulation of the transcriptional pattern at the *APOE* locus by DNA methylation.[43] The associations we found of *APOE* genotype with lipid fractions align well with those in a prior European study.[33] We investigated circulating lipid fraction concentrations as a potential underlying mechanism of prolonged survival in *APOE*-ε2 carriers, but found no evidence of mediation. In addition, estimates changed only minimally after taking into account clinically manifest vascular disease. Although this may in part reflect limitations of single measurements of lipid fractions, it also suggests other mechanisms are involved.[44]

Certain additional limitations should be taken into account. First, although we determined *APOE* genotype directly rather than by imputation, we did not investigate other genetic variants that might modify the effect of *APOE* through epistatic interactions. Second, Friedewald's formula for computation of LDL cholesterol assumes that all triglycerides are carried on VLDL, and a constant triglyceride-to-cholesterol-ratio of 5:1, which may not always apply. Third, we cannot rule out selection bias at older ages, which may affect ε4 carriers in particular. Fifth, albeit the largest study of *APOE*-ε2 in relation to mortality to date, precision may still be lacking with respect to separate genotypes to fully reveal a dose-effect response. Finally, the study population was entirely of European ancestry, and findings may not be applicable to other ethnicities.

In conclusion, *E2-CHARGE* brings together data from several population-based studies worldwide. In the first analysis of the data, we find that *APOE*-ε2 prolongs survival in the general population of European descent, which appears only in part explained by commonly determined lipid fractions, or prevalent vascular disease. Further studies are needed to determine the role of *APOE*-ε2 in vascular and other types of disease, above and beyond the absence of the *APOE*-ε4 allele. Various other population studies collect data on *APOE* genotype and disease outcomes, and inclusion of these data – in particular from ethnically diverse populations – may aid in elucidating the role of *APOE*-ε2 in health and disease.

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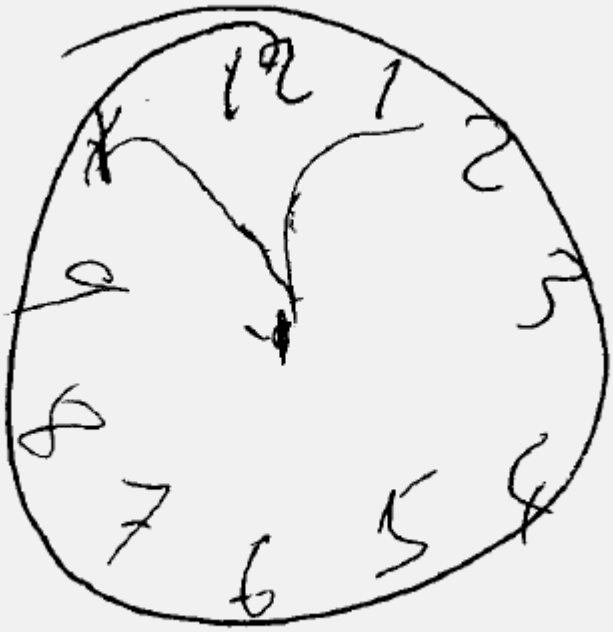
Chapter 5.2

APOE for trial design



ABSTRACT

Various clinical trials now aim to include individuals at high risk of dementia using genetic data, which increases the need for accurate risk prediction to inform study design and enrolment. However, available risk estimates are sparse, and the impact of the source population on absolute short-term estimates largely unexplored. To determine the risk of mild cognitive impairment (MCI) or dementia by *APOE*-ε4 dose, and identify potential risk modifiers, we included cognitively healthy individuals aged 60-75 years from four different cohorts, namely the National Alzheimer's Coordinating Center (NACC, *N*=5073), the Rotterdam Study (*N*=6399), the Framingham Heart Study (*N*=4078), and the Sacramento Area Latino Study on Aging (SALSA, *N*=1294). We computed stratified cumulative incidence curves for MCI and/or dementia by age (60-64, 65-69, 70-75 years) and *APOE*-ε4 dose, accounting for the competing risk of mortality, and assessed sex, education, family history, vascular risk, and baseline cognitive function as potential risk modifiers. Overall, cumulative incidence was uniformly higher in NACC than in the population-based cohorts. Among *APOE*-ε44 individuals, five-year cumulative incidence of MCI/dementia in the 60-64 age stratum was 0-6% in the three population-based cohorts versus 23% in NACC; in the 65-69 age stratum 9-10% versus 35%; and in the 70-75 age stratum 19-33% versus 38%. Five-year incidence of dementia was negligible except for *APOE*-ε44 individuals and those over 70. Differences of similar magnitude were seen between NACC and the population-based cohorts for heterozygous ε4 carriers. Lifetime incidence (to age 80-85) of dementia in the long-term Framingham and Rotterdam cohorts was 35% for the homozygous and 15% for heterozygous *APOE*-ε4 carriers, equal across baseline age groups. Confidence limits were often wide, particularly for *APOE*-ε44 individuals and for the dementia outcome at five years. In regression models, lower educational attainment, subjective memory concerns, worse cognitive performance at baseline, and family history of dementia consistently increased dementia risk. In conclusion, absolute risk estimates of MCI or dementia, particularly over short time intervals, are sensitive to sampling and a variety of methodological factors. Absolute risks are fairly consistent across population-based cohorts, but much higher in a convenience cohort, which has implications for informed consent and design for clinical trials targeting high-risk individuals.

INTRODUCTION

At present, 48 million people worldwide have dementia, and this number is projected to increase to 131 million by 2050.[1] Consequently, prevention of Alzheimer's disease, the most common type of dementia, has become a major research focus, with several prevention trials now underway.[2-4] The feasibility of such trials largely depends on the ability to recruit individuals at risk of developing disease during the trial period. One strategy to achieve this is to focus on individuals at high genetic risk. The Alzheimer Prevention Initiative[5] is embarking on two clinical trials targeting cognitively unimpaired individuals at highest genetic risk for Alzheimer's disease: one trial in an extended early-onset Colombian kindred carrying a fully penetrant presenilin 1 mutation (NCT01998841), and the Generation Study (NCT02565511), a trial in individuals ages 60-75 who carry two copies of the high-risk apolipoprotein E ε4 allele (*APOE*-ε4). The Generation Study is a double blind, randomized, placebo-controlled clinical trial of two different anti-amyloid agents in approximately 1,300 participants. Recruitment is through several sources, notably in the United States through the GeneMatch Alzheimer prevention registry.[6] High volume recruitment efforts are required because the *APOE*-ε44 genotype occurs in approximately 1-2% of the general population, so thousands of individuals must be screened to identify eligible participants. An assessment of absolute risk among eligible individuals in a meaningful time frame is essential for the informed consent process, as well as trial design, but in spite of numerous studies documenting *relative* risk increases for *APOE*-ε4 carriers (from 2- to 4-fold increases for heterozygous, to 8- to 15-fold for homozygous ε4 carriers),[7-11] the *absolute* risks are less clear.

When this study was initiated, available estimates of absolute risk of dementia for *APOE*-ε4 carriers were largely based on models developed from relative risks observed in one population and incidence data from another, often from case control samples. The Risk Evaluation and Education for Alzheimer's Disease Study (REVEAL) developed risk estimates based on observed absolute risks in first degree relatives versus spouses in a family sample,[12,13] and then applied relative risks by age, sex, and genotype from a large meta-analysis.[11] A more recent effort,[14] also reported on the 23andMe website,[15] applied relative risks from a recent European GWAS sample[16] to incidence estimates from the Rochester[17] and Personnes Agées Quid (PAQUID)[18] cohorts to compute lifetime risks by *APOE* genotype. Since that time, estimates from a single convenience cohort have been published, also with also high incidence rates.[19]

Because the available estimates of *APOE*-associated incidence of MCI or dementia were primarily based on models of disease onset rather than prospective observations, and

because *APOE* also affects longevity and risk for diseases other than dementia, we developed new estimates in population-based cohorts to better inform both trial designers and potential participants. For potential Generation Trial participants, the outreach and recruitment protocol for those who do not know their *APOE* genotype includes IRB-approved processes for obtaining their genotype and inviting them to a trial site. To ensure an appropriate disclosure setting during trial enrolment, prospective participants with and without the *APOE*-ε44 genotype are invited to assess trial eligibility and appropriateness for genetic disclosure visits. Our aims were to use prospective data to determine five-year and lifetime risk of MCI or dementia by age and *APOE*-ε4 dose among those as similar as possible to eligible trial participants (age 60-75, normal cognition) and to identify sources of heterogeneity that may account for variation in risk across populations.

METHODS

Study population

We sought available data from longitudinal population-based cohorts based on the following attributes: recruitment and a baseline cognitive evaluation at or before age 60, ongoing surveillance for assessment for MCI and dementia, and available *APOE* genotypes. Many ageing-focused cohorts (e.g., the Religious Orders Study,[20] the Cache County Study[21]) did not meet these criteria because of initial ascertainment at older ages. We also sought as broad ethnic representation as possible: we were able to include one Hispanic population with limited sample size, but no African American cohort was available with the requisite data. Three population-based cohorts were analysed: the Framingham Heart Study,[22] the Rotterdam Study,[23] and the Sacramento Area Latino Study on Aging (SALSA).[24,25] For comparison, we also included a longitudinal convenience cohort from the National Alzheimer's Disease Coordinating Center (NACC),[26] from the United States' multisite National Institute on Aging-funded Alzheimer's Disease Center Program), because we believed that NACC participants might resemble those volunteering for the trial in terms of key demographic variables and level of research interest.

Within each cohort, we selected participants with known *APOE* genotype who were cognitively unimpaired at the time of their first visit within the 60-75 years age window, and included all available subsequent visit information until diagnosis of MCI or dementia. For the two longer-term studies, the Framingham Heart Study and Rotterdam Study, individuals could contribute to multiple age strata for the stratified analyses, but were only included once in our regression analyses (see Statistical Analysis below). *APOE* genotype was measured in 94.1% (Rotterdam Study), 68.5% (Framingham Heart Study), 76.1% (NACC), and

92.0%% (SALSA) of otherwise eligible study participants, and only these individuals were included in the current study. On average, individuals who did not have their *APOE* genotype determined were slightly older in all cohorts but the Framingham Heart Study, in which they were younger. Those without *APOE* status were also more likely female in NACC and the Rotterdam Study, but more likely male in SALSA and the Framingham Heart Study. However, differences were generally small.

Ascertainment and assessment methods for each cohort

The original Framingham Heart Study cohort was recruited in 1948-1953 based on residence in Framingham, Massachusetts for a longitudinal study of cardiovascular disease (mean age at enrolment 45 years). A cohort of offspring of the original participants and their spouses was established in 1971-1975 (mean age at enrolment 37). Details of study procedures have been published elsewhere.[22] Cognitive status has been monitored in the original cohort since 1975, when a comprehensive neuropsychological battery was administered, followed by neurological assessment of participants with lower cognitive scores.[27] Since 1981, this cohort has been assessed at each examination with a Mini-Mental State Examination (MMSE), where participants were flagged for further cognitive screening if they scored below predefined education- and prior performance-based cut-offs. The offspring Cohort has undergone similar monitoring with serial MMSEs since 1991. Participants identified as having possible cognitive impairment based on these screening assessments (or in reports of cognitive concerns by the participant, family, treating physician, Framingham ancillary study investigators, or through review of outside medical records) are invited to undergo additional annual neurological and neuropsychological examinations. A dementia review panel including a neurologist and a neuropsychologist reviews each case of possible cognitive decline and dementia and categorizes participants based on the best available information (from serial neurological and neuropsychological assessments, telephone interviews with caregivers, medical records, neuroimaging, and, when available, autopsy data) and assigns a diagnosis and onset date for dementia according to DSM-IV criteria and MCI based on criteria by Petersen et al.[28] Diagnoses made prior to 2001 have been re-reviewed to update diagnostic criteria. Participants who entered the sample for the present analyses at a visit prior to MMSE administration but were cognitively unimpaired at subsequent study visits had this designation extended back to their earlier visits. For our regression analyses, these individuals were included at their first MMSE administration within our age window.

For the Rotterdam Study, individuals over 55 years in 1990 residing in a specific district of the City of Rotterdam, the Netherlands were invited to participate, with additional waves invited in 2000 (age >55 years) and 2005 (age >45 years). Details of study procedures have

previously been published.[23] In brief, all participants were interviewed at home and examined at the study centre every 4 to 5 years. Participants were routinely screened for dementia at baseline and follow-up examinations using the MMSE and the Geriatric Mental State Schedule (GMS).[29] Those with MMSE<26 or GMS>0 subsequently underwent an examination and informant interview using the Cambridge Examination for Mental Disorders of the Elderly.[30] Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records. The current sample included all participants with MMSE >26 at time of their first visit within the age window of interest. Formal assessment of MCI did not begin until 2005 in the Rotterdam Study. For the present analyses we therefore developed a pragmatic diagnosis of MCI, requiring a MMSE score <26 or a drop of at least 3 points from baseline, plus indicating memory concerns in a standardised questionnaire.

For SALSA, participants over 60 were sampled from six counties including census tracts with at least 5% Hispanic population in the Sacramento Valley of California in 1998-1999 and were followed approximately every 12-15 months until 2008. Detailed methods are described elsewhere.[24,31] In brief, dementia assessment included screening with both the Modified Mini-Mental State Examination (3MSE) and a word list learning task from a standard battery.[25,32] Those scoring below the 20th percentile (using age, education, sex, and language adjusted norms) on either test (or for follow-up visits, dropping 3 points in word list learning) were further evaluated using the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE),[33,34] and if this gave additional support for decline, were evaluated by a neurologist and categorized as cognitively unimpaired, memory-impaired (based on testing alone, without IQCODE corroboration), Cognitively Impaired Not Demented (CIND),[35] or dementia. Given the requirement for both a cognitive testing abnormality and confirmation from an informant, CIND was treated as equivalent to MCI.[31]

Participants in the NACC cohort were volunteers ascertained from various sources at 34 Alzheimer's Disease Centers in the United States. We used the March 2016 data freeze for the present analyses, so these data reflect study visits between September 2005 and March 2016. The participants were evaluated according to a standardized protocol,[36] with each subject and a collateral informant interviewed by the study clinician to rate the Clinical Dementia Rating (CDR),[37] plus a battery of neuropsychological tests.[38] A diagnosis was made at each visit by the study clinician following procedures at each site, and there were no study-wide standardized cut-offs on the CDR, MMSE, or other neuropsychological tests. Follow-up visits were conducted approximately annually.

Definition of predictor variables

Education was reported in years for SALSA and NACC and in categories of less than high school, high school, some college, or college graduation for Rotterdam and FHS. SALSA and NACC were translated into these categories as follows: <12 years: less than high school, 12 years: high school, 13-15 years: some college, and ≥16 years: college graduation. To assess general cognition across cohorts, we used MMSE for the Rotterdam Study, the Framingham Heart Study, and NACC, and 3MS for SALSA. To enable comparisons on relative performance within each cohort, we standardized based on the score at the baseline visit within each cohort, centring the raw scores around their sample mean and then dividing the centred scores by their standard deviation. Memory concerns at NACC were based on a single clinician-rated variable asking whether the subject believes that he or she has a problem with memory. Memory concerns in the Rotterdam Study were based on three questionnaire items asking 1) whether the participant is worried about his or her memory; 2) whether the participant ever loses track of what he or she is doing in the midst of an activity; and 3) whether the participant experiences word-finding difficulties. A positive answer to any of these questions qualified as memory concerns. Family history was defined as having at least one parent with dementia for the Rotterdam Study, and at least one first degree relative with dementia for NACC.

Analysis

We performed all analyses first for *MCI or dementia (*"MCI/dementia"), then for *dementia* alone. For the purposes of this trial, the MCI/dementia outcome was critically relevant, in that incident dementia was unlikely during the trial period, while there was tangible risk for MCI. Analyses for dementia only were performed as well because dementia is a more robust outcome than MCI.

We estimated *five-year* and "*lifetime*" (i.e., to age 80-85) cumulative incidence by *APOE*-ε4 dose and 5-year age baseline strata (age 60-64, 65-69, 70-75 years). We chose three age strata as a trade-off between addressing the steeply changing risk with age and the limited numbers of *APOE*-ε44 homozygotes, which left the ε-44 strata too small for stable estimates in the SALSA cohort. For the stratified analyses of the two longer-term studies, the Framingham Heart Study and the Rotterdam Study, individuals could contribute to multiple baseline age strata; we used the first visit within that age window as a baseline in these analyses. "Lifetime" estimates were computed as 20-year cumulative incidence for the age 60-64 stratum, as 15-year for the 65-69 stratum, and as 10-year for the 70-75 stratum; these estimates were only computed for the two longer-term studies to minimize extrapolation.

Stratified cumulative incidence curves by age stratum and *APOE*-ε4 dose were estimated,

adjusting for the competing risk of mortality.[42] In the presence of competing risks, the naïve Kaplan-Meier estimator, which treats the failure from the competing causes as censored observations, overestimates the cumulative incidence of the event of interest.[43] We used the 'cmprsk' package in R software to estimate the cumulative incidence for each age by *APOE*-ε44 dose stratum.[44] Following the suggestion of Lin,[45] we used the transformation *log[-log(1-x)]* to construct the confidence interval for cumulative incidence. The transformation not only ensures that the boundaries of cumulative incidence are contained in [0,1], but also improves the coverage accuracy.[45]

We used the same competing risks analytic framework to assess the effects of age and *APOE*-ε4 dose plus additional covariates on the cumulative incidence of MCI/dementia in order to inform personalized risk assessment and to understand differences across the cohorts. We used subdistribution hazard regression models,[46] because they directly link the regression coefficients with the cumulative incidence function (in contrast to cause-specific hazards regression, where the direct link cannot be made).[47,48] These analyses were also performed using the 'cmprsk' package in R software.[44]

For each cohort and for each outcome, we first fit univariable models for baseline age, sex, *APOE*-ε4 dose, education, standardized cognitive screen, subjective memory concerns, and family history of dementia. Then, we ran simple multivariable models for each outcome, including only *APOE*-ε4 dose and demographic factors (age, sex, and education). Last, we ran larger multivariable models also including standardized cognitive screen plus subjective memory concerns and family history of dementia if available for the cohort. Missing data on covariates were imputed using the mean of a 5-fold multiple imputation for analysis (Rotterdam Study: 11.5% for family history, 1% for educational attainment).

For the Rotterdam Study, the exact date of dementia diagnosis was used if available, and alternatively the midpoint of the interval between visits was used as the onset time of MCI or dementia at a study visit (conducted at four-year intervals) for both cumulative incidence estimates and subdistribution hazard regression. In addition, as a sensitivity analysis, we repeated our survival curves and regression models treating the onset of MCI or dementia as interval censored in addition to adjusting for the competing risk using the 'MIICD' package in R software to estimate the cumulative incidence, and results were extremely similar except for somewhat larger confidence intervals.

Unlike the stratified analyses, for the regression analyses, each subject was used only once. Typically, the first visit was the first visit within the eligible age window of 60-75. For the Framingham Heart Study, MMSE was not available at baseline visits prior to 1981 (as

described above). Thus, for the regression analyses, we reset the baseline visit as the first visit at which MMSE was available. This had the additional benefit of increasing the range of baseline ages within the cohort (see Table 1).

Meta-analyses were conducted for the five-year cumulative incidence estimates for all four cohorts and then for only the three population-based cohorts. Meta-analyses could not be conducted for the "lifetime" estimates because they only included two cohorts. As there was considerable heterogeneity among the studies, a random-effects meta-analysis based on DerSimonian-Laird method was used.[49] This analysis was performed using the 'metafor' package in R software. Because the primary goal was estimating cumulative incidence and understanding differences across cohorts and individuals rather than hypothesis testing, these analyses are reported with confidence intervals rather than statistical significance, and no adjustments are made for multiple comparisons.

Characteristics	**NACC** (n=5073)	**RS** (n=6399)	**FHS** (n=4078)	**SALSA** (n=1294)
Age at baseline, years	68.7 (4.3)	65.4 (4.2)	62.0 (1.7)	67.8 (4.4)
Men	1707 (33.6%)	2893 (45.2%)	1762 (43.2%)	538 (41.6%)
Education, years	15.8 (3.0)	12.9[#]	13.2[#]	7.7 (5.4)
No high school	140 (2.8%)	728 (11.4%)	622 (15.3%)	835 (64.5%)
High school	720 (14.2%)	2773 (43.3%)	1330 (32.6%)	201 (15.5%)
Some college	815 (16.1%)	1965 (30.7%)	1004 (24.6%)	126 (9.7%)
College graduation	3379 (66.6%)	871 (13.6%)	994 (24.4%)	125 (9.7%)
APOE genotype				
ε2/ε2, ε2/ε3, ε3/ε3	3431 (67.6%)	4598 (71.9%)	3166 (77.6%)	1112 (85.9%)
ε2/ε4, ε3/ε4	1484 (29.3%)	1645 (25.7%)	845 (20.7%)	171 (13.2%)
ε4/ε4	158 (3.1%)	156 (2.4%)	67 (1.6%)	11 (0.9%)
Family history of dementia	2957 (58.3%)	1191 (18.6%)	n/a	n/a
Cognitive screen score (MMSE or 3MS)[‡]	29.0 (1.3)	28.5 (1.0)	28.8 (1.4)	86.5 (11.3)
Subjective memory concerns	1262 (24.9%)	2759 (43.1%)	n/a	n/a
MCI or dementia during follow-up	602 (11.9%)	1301 (20.3%)	826 (20.3%)	111 (8.6%)
Dementia during follow-up	55 (1.1%)	782 (12.2%)	658 (16.1%)	49 (3.8%)
Remaining at 5-years of follow up	1865 (36.7%)	5592 (87.4%)	3911 (95.9%)	976 (75.5%)
Length of follow-up, years	3.96 (2.97)	12.64 (6.14)	17.59 (9.09)	6.50 (2.53)

Table 1. Population characteristics of subjects from the National Alzheimer Coordinating Center (NACC), the Rotterdam Study (RS), the Framingham Heart Study (FHS), and the Sacramento Area Latino Study on Aging (SALSA). Values are depicted as mean ±SD for continuous variables, and absolute numbers (%) for categorical variables. #In the Framingham Heart Study and the Rotterdam Study, educational attainment was recorded as categories. To facilitate comparisons with other samples, the mean was estimated by counting less than high school as 10 years, high school as 12, some college as 14, and college graduate as 16. Conversely, these values were used to assign categories to education for the NACC and SALSA cohorts. ‡ The Mini–Mental State Examination (MMSE) ranges from 0-30, whereas scores on the Modified Mini-Mental State Examination (3MS) range from 0-100. N/A=not available.

RESULTS

Table 1 presents the composition of the four cohorts. The cohorts differed considerably in size and duration of follow-up, with SALSA much smaller than the other cohorts, and long-term follow-up only available in the Framingham Heart Study and Rotterdam Study. Other differences were seen in educational attainment, with mean years ranging from less than 8 in SALSA to nearly 16 in NACC, and sex, with 34% men in NACC compared to 42-45% in the population-based cohorts. The cohorts also differed markedly in *APOE*-ε4 allele frequency, ranging from 7.5% in SALSA to 17.8% in NACC. NACC also had a 58.3% fraction with a family history of dementia, compared to 18.6% in Rotterdam, the only other site that assessed it.

Figure 1 shows the cumulative incidence of MCI/dementia stratified by baseline age group and *APOE*-ε4 dose. All four figures show 8.5 years of follow up on the same scale to facilitate comparison. Table 2 shows the corresponding five-year cumulative incidence of MCI/dementia for all cohorts, and for dementia alone. Figure 2 (MCI/dementia) and Table 3 (also for dementia alone) display the lifetime cumulative incidence (to age 80-85) across the two longer-term cohorts. Overall, within each cohort, risk increased with age and *APOE*-ε4 dose. However, absolute risks differed substantially across cohorts, particularly between NACC and the population-based cohorts. NACC typically had higher risk for any genotype at any age. Estimates among the population-based cohorts were very similar, particularly for longer-term follow-up and the dementia outcome.

Five-year cumulative incidence of MCI/dementia was low in the youngest age stratum, particularly in the cohort studies, although somewhat higher for *APOE*-ε4-positive individuals, especially homozygotes (23% in NACC versus 5-6% in Framingham and Rotterdam). Five-year incidence of MCI/dementia was higher in the highest age stratum, particularly among homozygotes (38% in NACC versus 18-23% in Framingham and Rotterdam). Five-year incidence of dementia alone was negligible at younger ages, even in *APOE*-ε44 homozygotes, but rose among older individuals, particularly homozygotes (12% in NACC versus 7-12% in Framingham and the Rotterdam). The meta-analyses of the five-year cumulative incidence estimates for the MCI/dementia outcome showed consistent increases in incidence by gene dose within age strata and by age stratum within gene dose, higher when the NACC estimates are included, ranging from a low of 1% for age 60-64 with no copies of *APOE*-ε4 in the population-based cohorts to a high of 27% for age 70-75 homozygotes with all four cohorts (Table 2). Estimated only for the Rotterdam Study and the Framingham Heart Study, lifetime incidence was consistent in the two cohorts and across age strata. For example, lifetime incidence of MCI/dementia rises with *APOE*-ε4 dose from 12-15% for those with no copies of *APOE*-ε4 to 37-47% for homozygotes (Table 3).

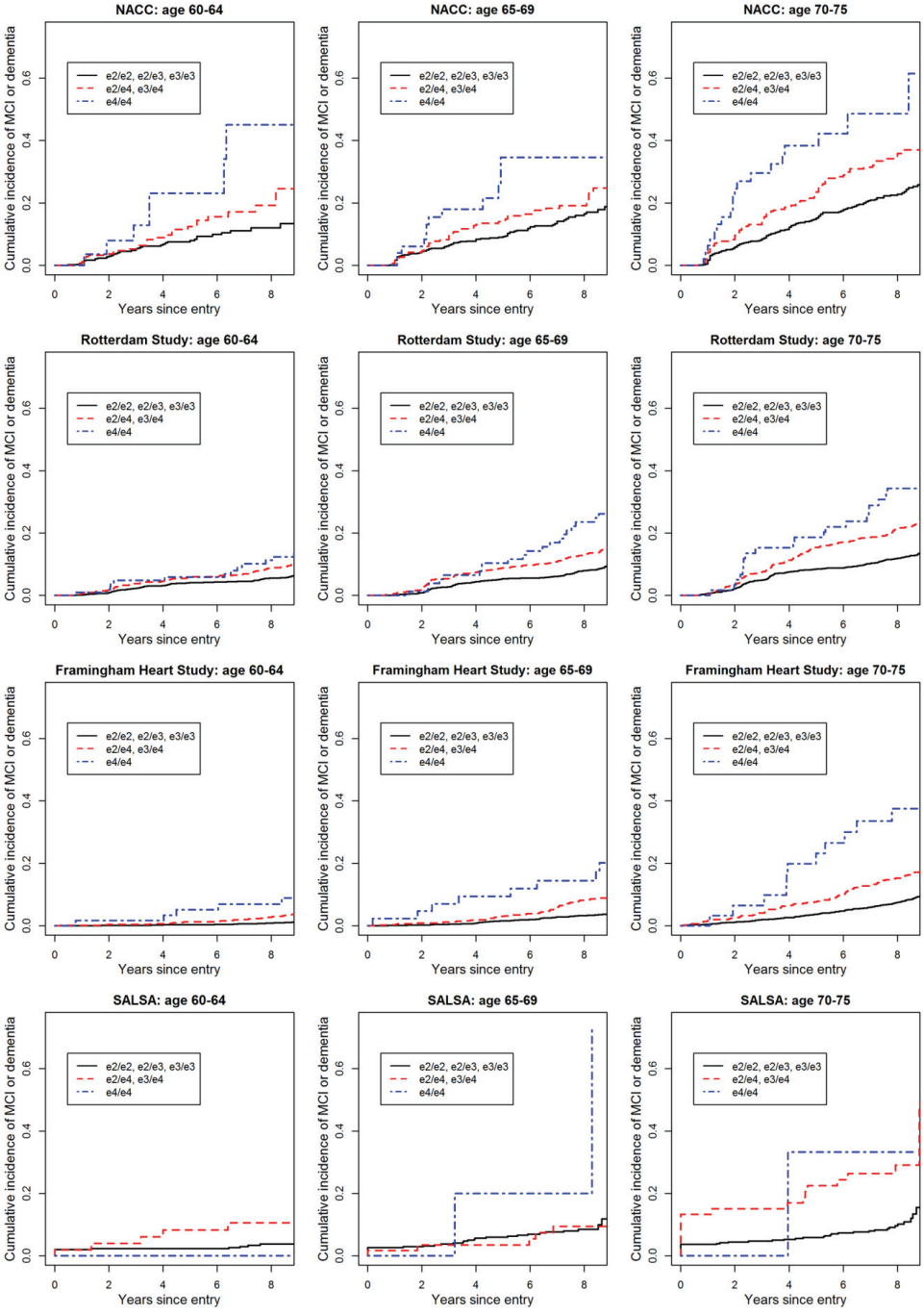


Figure 1. Cumulative incidence of MCI/dementia per cohort, stratified by age and *APOE* genotype.

MCI OR DEMENTIA	NACC		Rotterdam Study		Framingham		SALSA		Population-based	All cohorts
	N	5-year risk (95% CI)	*N*	5-year risk (95% CI)	*N*	5-year risk (95% CI)	*N*	5-year risk (95% CI)	5-year risk (95% CI)	5-year risk (95% CI)
Age 60-64										
Non-carrier ε4	740	7.9 (5.7-11.1)	2625	4.1 (3.4-4.9)	2955	0.3 (0.2-0.6)	352	2.3 (1.2-4.5)	1.5 (0.3-6.7)	2.3 (0.8-6.2)
Heterozygote ε4	322	12.4 (8.1-18.6)	928	5.7 (4.4-7.4)	795	1.3 (0.7-2.4)	51	8.3 (3.2-20.8)	3.9 (1.3-11.0)	5.3 (2.2-12.1)
Homozygote ε4	36	23.1 (9.9-48.1)	102	5.9 (2.7-12.7)	62	5.1 (1.7-15.0)	3	0	5.6 (2.9-10.5)	9.1 (3.4-23.2)
Age 65-69										
Non-carrier ε4	1172	9.2 (7.3-11.6)	2492	5.4 (4.6-6.3)	2430	1.5 (1.1-2.1)	377	6.0 (3.9-9.0)	3.6 (1.3-9.6)	4.6 (1.9-11.2)
Heterozygote ε4	585	14.4 (11.1-18.6)	906	8.8 (7.2-10.9)	646	3.0 (1.9-4.7)	60	3.5 (0.9-13.3)	4.8 (2.0-11.7)	6.8 (3.5-13.0)
Homozygote ε4	65	34.6 (20.2-55.2)	77	10.4 (5.3-19.8)	44	9.4 (3.6-23.5)	5	20.0 (2.5-86.5)	10.5 (6.2-17.6)	16.4 (7.3-34.5)
Age 70-75										
Non-carrier ε4	1519	15.2 (13.0-17.6)	2212	8.4 (7.3-9.7)	1888	3.7 (2.9-4.7)	383	5.9 (3.9-8.8)	5.7 (3.2-10.1)	7.4 (4.1-13.2)
Heterozygote ε4	577	23.6 (19.5-28.3)	739	15.5 (13.0-18.3)	464	7.7 (5.6-10.6)	60	22.5 (13.6-35.8)	13.7 (7.9-23.1)	15.9 (10.0-24.6)
Homozygote ε4	57	38.3 (25.1-55.5)	59	18.6 (10.7-31.3)	32	23.2 (11.6-43.0)	3	33.3 (2.7-99.8)	20.6 (13.5-30.7)	26.7 (17.5-39.5)
DEMENTIA	*N*	5-year risk (95% CI)	*N*	5-year risk (95% CI)	*N*	5-year risk (95% CI)	*N*	5-year risk (95% CI)	5-year risk (95% CI)	5-year risk (95% CI)
Age 60-64										
Non-carrier ε4	740	0	2625	0.2 (0.1-0.4)	2955	0.03 (0.0-0.3)	352	0.6 (0.1-2.3)	0.2 (0.04-0.6)	0.2 (0.04-0.6)
Heterozygote ε4	322	0.5 (0.1-3.8)	928	0.5 (0.2-1.3)	795	0	51	2.0 (0.3-13.4)	0.8 (0.3-2.3)	0.7 (0.3-1.4)
Homozygote ε4	36	0	102	2.9 (1.0-8.9)	62	0	3	0	2.9 (1.0-8.9)	2.9 (1.0-8.9)
Age 65-69										
Non-carrier ε4	1172	0.4 (0.1-1.2)	2492	0.5 (0.3-0.9)	2430	0.3 (0.1-0.6)	377	0.9 (0.3-2.8)	0.5 (0.3-0.9)	0.5 (0.3-0.7)
Heterozygote ε4	585	1.0 (0.3-3.0)	906	0.9 (0.4-1.8)	646	0.6 (0.2-1.7)	60	3.5 (0.9-13.3)	1.1 (0.5-2.4)	1.0 (0.6-1.8)
Homozygote ε4	65	4.4 (1.1-16.6)	77	5.2 (2.0-13.3)	44	4.8 (1.2-18.0)	5	0	5.1 (2.3-11.0)	4.9 (2.5-9.6)
Age 70-75										
Non-carrier ε4	1519	1.4 (0.8-2.5)	2212	1.5 (1.0-2.0)	1888	1.0 (0.6-1.6)	383	2.6 (1.4-5.0)	1.5 (0.9-2.3)	1.4 (1.0-2.0)
Heterozygote ε4	577	3.0 (1.6-5.6)	739	6.5 (4.9-8.5)	464	3.1 (1.8-5.2)	60	6.8 (2.6-17.2)	5.1 (2.9-8.7)	4.5 (2.8-7.2)
Homozygote ε4	57	12.4 (5.3-27.9)	59	11.9 (5.8-23.4)	32	6.7 (1.7-24.6)	3	0	10.5 (5.6-19.3)	11.1 (6.7-18.2)

Table 2. Five-year cumulative incidence of MCI/dementia (top) and dementia only (bottom) by baseline age and *APOE* genotype.

	Mild cognitive impairment or dementia				Dementia			
	Rotterdam Study		Framingham		Rotterdam Study		Framingham	
	N	Lifetime risk* (95% CI)	*N*	Lifetime risk* (95% CI)	*N*	Lifetime risk* (95% CI)	*N*	Lifetime risk* (95% CI)
Age 60-64								
Non-carrier ε4	2625	14.1 (12.5-15.8)	2955	11.9 (10.6-13.5)	2625	6.8 (5.6-8.3)	2955	6.2 (5.2-7.4)
Heterozygote ε4	928	25.4 (22.0-29.2)	795	22.1 (18.7-26.0)	928	17.2 (14.1-20.9)	795	15.9 (13.0-19.5)
Homozygote ε4	102	37.5 (25.1-53.3)	62	45.2 (31.3-61.7)	102	34.7 (22.8-50.5)	62	38.5 (25.5-55.2)
Age 65-69								
Non-carrier ε4	2492	14.2 (12.8-15.8)	2430	12.2 (10.8-13.8)	2492	5.3 (4.4-6.3)	2430	6.6 (5.5-7.8)
Heterozygote ε4	906	25.4 (22.5-28.7)	646	23.3 (19.8-27.4)	906	15.4 (12.9-18.2)	646	16.2 (13.2-19.9)
Homozygote ε4	77	38.1 (27.3-51.5)	44	46.7 (31.6-64.7)	77	30.8 (20.7-44.1)	44	40.3 (25.8-59.0)
Age 70-75								
Non-carrier ε4	2212	15.6 (14.1-17.2)	1888	11.9 (10.4-13.6)	2212	5.8 (4.9-6.8)	1888	5.7 (4.6-6.9)
Heterozygote ε4	739	26.1 (23.1-29.5)	464	21.4 (17.6-25.8)	739	14.8 (12.4-17.6)	464	13.9 (10.8-17.9)
Homozygote ε4	59	38.0 (26.8-52.0)	32	37.6 (22.4-58.2)	59	33.3 (22.5-47.4)	32	35.2 (20.3-56.3)

Table 3. Remaining lifetime risk (till age 80-85) of the composite MCI/dementia, and dementia alone, by baseline age and *APOE*-ε4 dose. * Lifetime risk is estimated as 20-year cumulative incidence for age 60-64, 15-year cumulative incidence for age 65-69, and 10-year cumulative incidence for age 70-75.

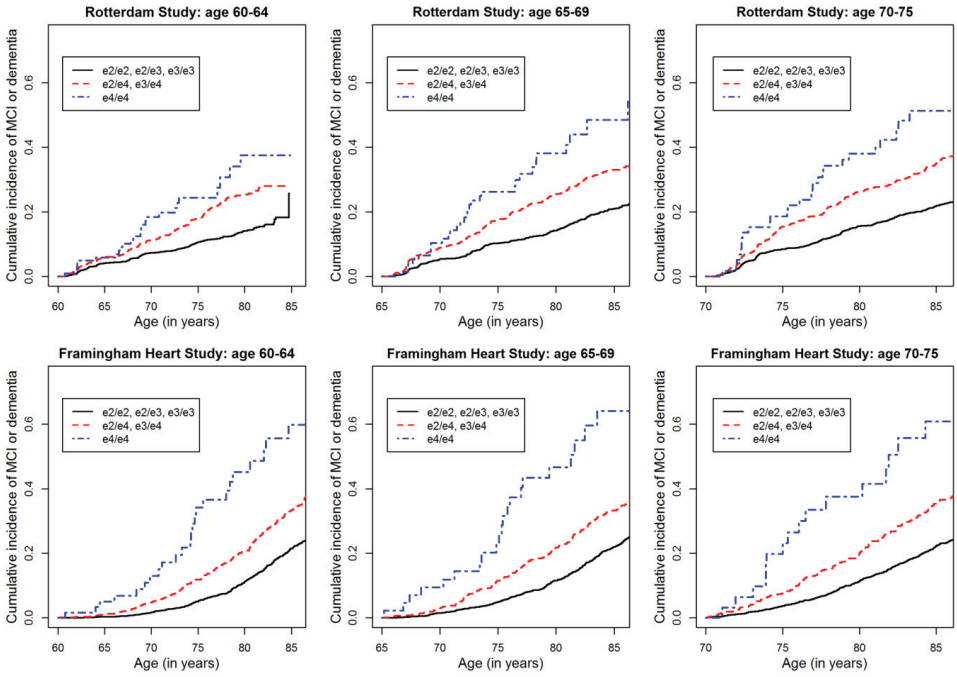


Figure 2. Lifetime risk of MCI/dementia in the two long-term cohorts.

Results of univariable and multivariable subdistribution hazard regression are presented in Table 4. Overall, the regression results were fairly consistent across the four cohorts, even in the small SALSA sample, and considerably more consistent than the cumulative incidence results.

The univariable results showed substantially higher risk with increasing age, increasing *APOE*-ε4 dose, and lower education. Family history also had a nominally significant effect in both cohorts in which it was measured. Men were at lower risk in the population-based cohorts, but carried higher risk in NACC. Risk estimates for sex were attenuated after adjustment for education. Subjective memory concerns carried risk in both cohorts that assessed them. Higher standardized baseline MMSE or 3MS score was consistently protective across all cohorts, except for Rotterdam. It is noteworthy that standardized cognitive screen performance and subjective memory concerns generally showed substantial hazard ratios, even controlling for education, and that family history, even when controlling for *APOE*-ε4 dose, also had an impact. Overall, we observed similar risk estimates for MCI/dementia and for dementia alone, although some estimates were slightly higher for the dementia outcome (data not shown).

	NACC* Hazard ratio (95% CI)	Rotterdam Study Hazard ratio (95% CI)	Framingham Hazard ratio (95% CI)	SALSA Hazard ratio (95% CI)
Age at baseline	1.08 (1.06-1.10)	1.09 (1.08-1.10)	1.16 (1.14-1.18)	1.07 (1.06-1.09)
Male sex	1.36 (1.16-1.60)	0.83 (0.74-0.93)	0.89 (0.76-1.04)	0.85 (0.64-1.13)
***APOE* genotype**				
Non-carrier ε4	Reference	Reference	Reference	Reference
Heterozygote ε4	1.51 (1.27-1.78)	1.64 (1.46-1.84)	1.57 (1.32-1.88)	2.03 (1.45-2.82)
Homozygote ε4	2.79 (1.98-3.92)	2.63 (2.02-3.42)	3.30 (2.08-5.22)	2.22 (0.75-6.57)
Education				
No high school	1.86 (1.24-2.80)	1.41 (1.21-1.64)	1.80 (1.46-2.22)	1.53 (0.99-2.36)
High school	Reference	Reference	Reference	Reference
Some college	0.83 (0.62-1.11)	0.81 (0.71-0.93)	1.05 (0.85-1.29)	0.70 (0.32-1.50)
College graduation	0.85 (0.67-1.06)	0.56 (0.46-0.69)	0.76 (0.60-0.96)	1.01 (0.51-1.97)
Cognitive screen (per standard deviation)‡	0.62 (0.57-0.66)	1.00 (0.95-1.06)	0.80 (0.76-0.85)	0.63 (0.58-0.68)
Subjective memory concerns	2.62 (2.22-3.08)	1.71 (1.53-1.91)	N/A	N/A
Family history of dementia	1.16 (0.98-1.37)	1.27 (1.11-1.44)	N/A	N/A
	Hazard ratio (95% CI)	Hazard ratio (95% CI)	Hazard ratio (95% CI)	Hazard ratio (95% CI)
Age at baseline	1.08 (1.05, 1.10)	1.08 (1.07, 1.09)	1.15 (1.12, 1.17)	1.07 (1.03, 1.12)
Male sex	1.14 (0.96,1.36)	0.92 (0.81, 1.03)	0.93 (0.79, 1.10)	0.84 (0.56,1.25)
***APOE* genotype**				
Non-carrier ε4	Reference	Reference	Reference	Reference
Heterozygote ε4	1.49 (1.25, 1.79)	1.63 (1.44, 1.84)	1.75 (1.45, 2.10)	2.15 (1.39, 3.33)
Homozygote ε4	2.37 (1.59, 3.53)	2.78 (2.10, 3.69)	4.01 (2.31, 6.96)	1.65 (0.27, 9.93)
Education				
No high school	1.41 (0.91, 2.19)	1.24 (1.06, 1.46)	1.33 (1.06, 1.65)	0.80 (0.43, 1.49)
High school	Reference	Reference	Reference	Reference
Some college	0.90 (0.66, 1.22)	0.83 (0.72, 0.95)	1.10 (0.89, 1.36)	1.01 (0.42, 2.43)
College graduation	0.92 (0.73, 1.16)	0.62 (0.50, 0.77)	0.87 (0.69, 1.11)	1.61 (0.72, 3.62)
Cognitive screen (per standard deviation)‡	0.63 (0.58, 0.69)	1.08 (1.02, 1.15)	0.87 (0.82, 0.93)	0.59 (0.52, 0.67)
Subjective memory concerns	2.23 (1.87, 2.66)	1.56 (1.39, 1.74)	N/A	N/A
Family history of dementia	1.27 (1.06, 1.52)	1.16 (1.01, 1.32)	N/A	N/A

Table 4. Regression for MCI/dementia. Results from univariable (top table) and multivariable analysis (bottom table) are presented per cohort. ‡Mini–Mental State Examination (MMSE) for NACC, the Rotterdam Study, and the Framingham Heart Study; and a Modified Mini-Mental State Examination (3MSE) in SALSA.

DISCUSSION

Of 16,844 participants included from all four cohorts, 392 (2.3%) were *APOE*-ε44 homozygotes, highlighting the low prevalence of this genotype. Nonetheless, the expected *APOE*-ε4 dose-related increases in cumulative incidence and relative hazard in the regression models are readily apparent. Striking differences in estimated cumulative incidence, however, between the population-based cohort studies and the highly ascertained NACC cohort suggest that for trial design and informed consent, exploratory efforts will be required to accurately match risk estimates to characteristics of the trial population.

Overall, *APOE*-ε4-associated incidence is somewhat lower in the presented cohorts than in models findings previously available in the literature, although NACC findings were largely similar to previous prospective analyses in the same cohort,[19] although that study focused mostly on the *relative* risk of APOE-ε4 across different age ranges, without incorporating other predictor variables, and did not perform their analysis in a competing risk framework, which is vital to avoid overestimation of cumulative incidences due to mortality. The three population cohorts were generally similar, within expected sampling variation, in their estimates of cumulative incidence for most age and *APOE* strata. The difference between the population-based cohort studies and NACC, on the other hand, is striking. Large variability in risk estimates related to ascertainment and assessment methods has been reported previously for MCI and dementia prevalence.[50,51] Such variability can occur in a variety of settings, but is a particular problem for **c**ommon disorders like MCI where a subtle gradation from the normal makes rates especially sensitive to thresholding (similar to for example Attention Deficit Hyperactivity Disorder, major depression, and osteoarthritis). As might be expected, *absolute* risk is much more vulnerable to methodological differences than *relative* risk, especially over shorter time intervals, and for the MCI/dementia outcome rather than the dementia alone outcome. This is underscored by the generally similar *relative hazards* across the regression analyses. Notwithstanding the NACC cohort is a volunteer cohort, and as such would not be expected to represent of the general population. Individuals join this cohort for a variety of reasons, of which concerns about family history and their own memory are likely to play a role, as evidence by the relatively high *APOE*-ε4 allele frequency. As family history increases risk beyond the *APOE*-ε4 effect,[52,53] this likely contributed to some of the observed differences in incidence. Another potential source of difference is the very high level of educational attainment within the NACC cohort. While higher education is associated with *lower* risk of dementia overall, among those with memory concerns, the risk has been seen to be *higher*,[54] and this may be particularly true for the highly educated individuals who form a substantial fraction of the NACC cohort. Another issue is the high proportion of women in the NACC cohort. Different reasons between sexes for volunteering

may underlie the *increased* risks for men in NACC, as opposed to the other cohorts. Last, within NACC there is substantial drop out and variable effort to retain subjects, and decisions by participants and centre staff are not likely to be random with respect to cognitive and other variables. While the population-based cohort studies also have some drop out, systematic ongoing efforts to retain subjects and continuous surveillance even for those who do not attend study visits guarantee low attrition. Beyond these differences in ascertainment, demographics, and follow-up, there are differences in assessment between NACC and the three population-based cohorts that should be noted. The population-based cohorts evaluate cognition with a screening procedure typically followed by more formal clinical evaluation of subjects who screen positive. While direct clinical evaluation of all participants at each NACC site is a strength, there are procedural differences across sites, quality control is limited, and the reliability of NACC diagnosis is not well established. Of course, it is likely that there is some insensitivity to MCI and even dementia in the population-based cohort studies, as well as differential loss to follow-up, but on balance the volunteer nature of the NACC cohort with limited quality control across sites, and the consistency of the population-based findings tend to favour the lower cumulative incidence.

One could argue that previously available modelled estimates for *APOE*-ε4-associated absolute risk for dementia are high (50-67%),[12,14] and thus favour the NACC estimates. Our estimates of lifetime risk for dementia for *APOE*-ε44 carriers from the Framingham Heart Study and the Rotterdam Study are in the 31-40% range. However, there are some biases in the previously modelled estimates that overall are more likely to yield over- rather than underestimates of risk. In the REVEAL Study,[12] risk curves for incidence were derived from relatives and spouses in a family sample ascertained from a clinical population.[13] These incidence rates are expected to be higher than those in the general population. In addition, the relative risks by sex, age, and genotype were applied from a large meta-analysis done primarily in clinically ascertained, younger onset families,[11] again yielding higher estimates.[11,55] The competing risk of death was furthermore not addressed in cumulative incidence estimates, which also would tend to bias estimates upward, and the applied models did not account for the correlation among observations in the family sample used for incidence, which again might lead to bias.[56] For the estimates used by 23 and Me,[14] relative risks from the European GWAS[16] were applied to incidence estimates from the Rochester,[17] and PAQUID cohorts.[18] The relative risk estimates come from cases and controls, with younger cases (with a greater *APOE*-ε4 effect) overrepresented. In addition, these models assumed that the controls in GWAS samples were representative of the overall population, which likely does not hold with a very common disease like dementia, because at higher ages those without dementia are fundamentally a selected sample. This also would tend to bias the estimates upward.

In the regression models, we observed consistent effects of age and *APOE*-ε4 dose across the univariable and multivariable models, persisting even when other demographic factors, cognitive variables, and family history were taken into account. Education also exhibited a dose response, but less consistently, as much illustrating as illuminating the profound differences in education across these four samples. Ascertainment and cultural differences across disparate samples may have contributed to sex differences. In the population-based cohorts, there was strong attenuation of the estimates of sex when adjusting for educational attainment, suggesting lack of education in women of older birth cohorts may partly explain the difference. Remarkably, in NACC risk was higher in men, which likely relates to ascertainment differences in this convenience sample, as noted above. Also of potential relevance, both to potential participants wishing to understand their absolute risk and to investigators designing clinical trials, both cognitive screen performance and subjective memory concerns are associated with an increased hazard of MCI or dementia. All in all, these associations suggest that relatively simple individual characteristics might be used to further refine individual risk stratification beyond age and *APOE* genotype.

Our findings have several implications for trial design and genetic counselling. For purposes of prevention trials, absolute cumulative incidence, both for the duration of the trial and over the remaining lifetime, are critical, but the differences across these cohort studies make it difficult to offer precise estimates, even with meta-analyses. In an ideal world, estimates would be tailored to the population entering the trial, or better still, the specific individuals, and would take into account not only explicit inclusion criteria but also any other measureable or predictable characteristics that might predict willingness to volunteer. A review of the first registrants on the GeneMatch Registry that serves as the primary US recruiting site for the Generation *APOE*-ε44 trial shows that individuals differ substantially beyond the explicit entry criteria. The population of 13,704 registrants enrolled thus far is relatively young (mean age 62.7, SD 5.2) and women are overrepresented (80%). Among the 4,978 registrants who were asked about race/ethnicity, 92% are white. *APOE*-ε44 genotype is higher than in the general population at 4.5%, corresponding to an *APOE*-ε4 allele frequency of 20.4%, and among the 3,456 registrants asked about family history of dementia or Alzheimer's, 70% said yes. While education was not measured, the high percent of females and familial predisposition suggests a population that may be more like NACC. Yet, over time, if broader recruiting efforts are applied to reach the target sample size, volunteers could gradually become more reflective of the general population, and lower risks might be expected. For genetic counselling, any risk information would need to give a broad range of estimates to reflect uncertainty within cohorts and variation across cohorts. Because risk for disease is ongoing beyond trial duration, and lifetime risks are more stable than short-term risks, these lifetime risk could be more informative for genetic disclosure.

However, such risks may be less salient to some of those considering enrolment in trials at younger ages. Relative risks by *APOE* genotype or *APOE*-ε4 dose have limited relevance, but may provide context. If these are provided, risk should be compared to the general population (based on a weighted average across *APOE* genotypes) rather than the typical "no *APOE*-ε4" base category used in regression models, which would more fairly allow a participant to put his or her own risk in the context of friends and acquaintances of unknown genotype. On the basis of our regression findings, for *APOE*-ε44 homozygotes, adjusted relative risks for MCI/dementia are 2.7 for NACC, 3.4 for Framingham, and 2.4 for Rotterdam, so disclosing a relative risk of about 3-fold compared to the general population would be sensible. Use of pictographs as a visual aid to risk communication could be useful, given their ability to visually represent both absolute and relative risk information simultaneously.[57] In addition, there is a robust literature on genetic risk communication that can inform best practice when *APOE* information is disclosed to asymptomatic individuals.[58]

A major limitation of the current study is that *APOE*-ε44 samples are small despite the large size of the initial cohorts, particularly for SALSA. This limits the stability of stratified cumulative incidence estimates, as well as regression coefficients for *APOE* dose. Second, while the four cohorts are heterogeneous in sex distribution and education, there is little ethnic and racial diversity, so the findings are less relevant to participants of non-European background. Third, variations in definitions of the exposure and outcome variables may hamper comparison among cohorts. As noted above, each sample uses different criteria to define unimpaired at baseline, and to screen, assess, and diagnose new onsets. Different psychometric tests are applied, and even the same test performs differently across different groups, which may be solved only in part by education and/or age-adjusted norms. Other variation may come from differences in definitions (e.g. family history) or in how information is acquired (e.g. memory concerns by questionnaire or overall clinical impression). Moreover, some variables, notably levels of education, may be defined similarly but have different meanings within different cultural contexts. Nevertheless, as we have shown, *relative* risk estimates are consistent despite this variation.

In conclusion, prospective cohort studies can be used to inform study design, power, and informed consent in clinical trials among cognitively healthy individuals. While trial designers and participants may be most interested in absolute risk over relatively short intervals, such estimations are less robust than long-term risks, and more susceptible to changes in demographic and clinical characteristics between populations. Informed consent and optimal trial design is therefore best served by matching eligible trial participants to available observational cohort studies as closely as possible, which will require exploratory efforts to accurately determine characteristics of the trial population.

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Chapter 5.3

Parental family history of dementia

ABSTRACT

Family history is an important risk factor for dementia, but its applicability for clinical risk stratification largely depends on the magnitude of the associated risk. Age at onset and sex of the affected relative have been shown important determinants of familial risk in other diseases, such as myocardial infarction and stroke, and while several small brain imaging studies do suggest preferential maternal transmission of susceptibility to neurodegeneration, no published studies have determined the risk of dementia by age- and sex-specific parental family history. Between 2000 and 2002, we assessed parental history of dementia in non-demented participants of the Rotterdam Study. We investigated associations of parental history with risk of dementia until 2015, adjusting for demographics, cardiovascular risk factors, and known genetic risk variants. Furthermore, we determined the association of parental history with markers of neurodegeneration and vascular disease on MRI. Of 2,087 participants (mean age 64 years, 55% female), 407 (19.6%) reported a history of dementia in either parent (mean age at diagnosis: 79 years). During a mean follow-up of 12.2 years, 142 participants developed dementia. Parental history was associated with risk of dementia independent of known genetic risk factors (hazard ratio [95% confidence interval]: 1.67 [1.12-2.48]), in particular when parents were diagnosed at younger age (<80 years: HR 2.58 [1.61-4.15] versus ≥80 years: 1.01 [0.58-1.77]). Accordingly, age at diagnosis in probands was highly correlated with age at diagnosis in their parents <80 years (r=0.57, P=0.001), but not thereafter (r=0.17,P=0.55). Among 1,161 non-demented participants with brain MRI, parental history related to lower cerebral perfusion, and higher burden of white matter lesions and microbleeds. Dementia risk and MRI markers were similar for paternal versus maternal history. In conclusion, enquiring age at parental diagnosis greatly enhances the value of taking a family history of dementia. Unexplained heredity is substantial, and may in part be attributed to cerebral hypoperfusion and small-vessel disease. We found no evidence of preferential maternal compared to paternal transmission.

INTRODUCTION

Family history of dementia is an important risk factor for dementia and Alzheimer's disease, independent of known genetic risk factors for Alzheimer's disease.[1] Yet, its applicability for clinical risk stratification and research about underlying mechanisms largely depends on the magnitude of the associated risk. For other diseases, such as myocardial infarction, the strength of associations between family history and risk of disease diminishes with increasing age at which family members are affected.[2] Similarly, with regard to dementia, the effect of its major genetic risk factor (*APOE*) as well as the heritability of brain morphology decline with age,[3,4] but prospective studies that quantify associations of family history with risk of dementia by age at onset of affected relatives are lacking.

In search of potential mechanisms that account for the unexplained heredity of dementia, several studies have recently turned to imaging markers of neurodegeneration. These generally explorative studies found that in healthy adults, a family history of dementia is associated with structural brain changes,[5-8] and various other markers of neurodegeneration, including white matter integrity,[9-10] resting state connectivity,[11] glucose metabolism,[12-15] hypoperfusion,[16] and β-amyloid and tau.[12,14,15] Interestingly, several of these studies have suggested a stronger association with maternal compared to paternal family history,[6,12-16] but this was not confirmed in two other reports.[5,7] Sex-specific transmission is plausible in view of findings for ischaemic stroke and myocardial infarction,[17,18] and may relate to chromosome X mutations, mitochondrial DNA, or imprinting.[19] However, no published studies have assessed risk of developing dementia by paternal and maternal history.

We therefore investigated the association of family history, by age at onset and sex of affected parent, with risk of dementia in the general population, and explored underlying imaging abnormalities on structural MRI.

METHODS

Study population

This study is embedded within the Rotterdam Study, a large ongoing population-based cohort study in the Netherlands among inhabitants aged ≥55 years from the Ommoord area in Rotterdam. For the current study, we included the second wave of invitees, recruited between 2000 and 2002. The Rotterdam Study methods have been described in detail previously.[20] In brief, participants were interviewed at home and subsequently examined at

the research centre from January 2000 to November 2002, which was used as baseline for this study. Family history of dementia was assessed during baseline interview. Of 3,011 eligible participants, 2,247 (74.6%) underwent home interview. From August 2005 until July 2013, surviving participants of the subsequent examination cycle were all invited for magnetic resonance imaging (MRI).

Assessment of family history

Participants were questioned by trained interviewers about parental family history of dementia, using a structured questionnaire. If this question was answered positively, they were further asked about specific paternal and maternal history of dementia, including age at diagnosis. Vital status of parents and age of death were also recorded.

Genotyping and calculation of genetic risk scores

DNA was extracted from blood samples drawn by venipuncture at baseline. *APOE* genotype was determined with a bi-allelic TaqMan assay (rs7412 and rs429358) in 97.9% of participants. The majority of samples (81.1%) were further genotyped using the Illumina 610K and 660K chip, and imputed to the Haplotype Reference Consortium reference panel (v1.0) with Minimac 3. We included 23 genetic variants that showed genome wide significant evidence of association with Alzheimer's disease to calculate a weighted genetic risk score (Table 1). The genetic risk score was calculated as the sum of the products of SNP dosages of the 23 genetic variants (excluding *APOE*) and their respective reported effect estimates. All 23 variants selected for the calculation of the genetic risk score were well imputed (imputation score R^2>0.3, median=0.99).

MRI scan protocol and image processing

Brain MRI was done on a 1.5 T scanner (GE Healthcare, Milwaukee, WI, USA), with use of an 8-channel head coil.[21] We acquired a high-resolution axial T1-weighted sequence, proton-density-weighted (PD) sequence, a fluid attenuated inversion recovery (FLAIR) sequence, and a T2*-weighted gradient echo sequence, as described previously.[21] Quantification of parenchymal volume and volume of white matter hyperintensities was done using an automated tissue segmentation method.[22] All segmentations were inspected and manually corrected if so required. All scans were appraised by trained research physicians, blinded to clinical data, for the presence of cerebral microbleeds and lacunar infarcts (i.e. focal lesions ≥3 and <15mm in size with similar signal intensity as cerebrospinal fluid on all sequences). Cerebral blood flow was determined from 2D phase-contrast images with custom software (Cinetool version 4; General Electric Healthcare).[23] We calculated total brain perfusion (in mL/min per 100 mL brain tissue) by dividing total blood flow (mL/min) by each individual's brain volume (mL) and multiplying the result by 100.

Chr	Rsid	ALT-HRC	Gene	Locus identified	Effect estimate	MAF	Weight ALT-HRC	R^2 RS1	R^2 RS2	R^2 RS3
19	rs4147929	G	ABCA7	Hollingworth (2011),[37] Naj (2011)[38]	Lambert (2013)[39]	0.19	-0.135	0.916	0.917	0.991
2	rs6733839	T	BIN1	Seshadri (2010)[40]	Lambert (2013)	0.409	0.188	0.960	0.911	0.962
20	rs7274581	C	CASS4	Lambert (2013)	Lambert (2013)	0.083	-0.139	0.990	0.989	0.990
6	rs1094836	G	CD2AP	Hollingworth (2011), Naj (2011)	Lambert (2013)	0.266	0.098	0.998	0.998	0.998
11	rs1083872	C	CELF1	Lambert (2013)	Lambert (2013)	0.316	0.075	0.998	0.998	0.998
8	rs9331896	T	CLU	Harold (2009),[41] Lambert (2009)[42]	Lambert (2013)	0.379	0.146	0.902	0.974	0.901
1	rs6656401	G	CR1	Lambert (2009)	Lambert (2013)	0.197	-0.157	0.953	0.948	0.950
10	rs7920721	G	ECHDC3	Desikan (2015)[43]	Desikan (2015)	0.3867	-0.029	1.000	1.000	1.000
7	rs1177114	A	EPHA1	Hollingworth (2011), Naj (2011)	Lambert (2013)	0.338	-0.102	0.998	0.998	0.999
14	rs1712594	C	FERMT2	Lambert (2013)	Lambert (2013)	0.092	0.122	1.000	1.000	1.000
6	rs1114182	A	HLA-	Lambert (2013)	Lambert (2013)	0.276	-0.108	0.314	0.312	0.314
4	rs1311369	G	HS3ST1	Desikan (2015)	Desikan (2015)	0.2825	-0.029	0.999	0.998	0.999
2	rs3534966	T	INPP5D	Lambert (2013)	Lambert (2013)	0.488	0.066	0.975	0.973	0.976
17	rs1181729	G	KANSL1	Jun (2016)[44]	Lambert (2013)	0.873	-0.151	0.710	0.700	0.708
5	rs190982	A	MEF2C	Lambert (2013)	Lambert (2013)	0.408	0.080	0.979	0.934	0.978
11	rs983392	G	MS4A6A	Hollingworth (2011), Naj (2011)	Lambert (2013)	0.403	-0.108	0.989	0.990	0.991
7	rs2718058	G	NME8	Lambert (2013)	Lambert (2013)	0.373	-0.070	1.000	1.000	1.000
11	rs1079283	G	PICALM	Harold (2009)	Lambert (2013)	0.358	0.130	0.999	0.999	0.999
8	rs2883497	C	PTK2B	Lambert (2013)	Lambert (2013)	0.366	0.096	0.993	0.990	0.994
14	rs1049863	T	SLC24A4-	Lambert (2013)	Lambert (2013)	0.217	-0.104	0.999	0.999	1.000
11	rs1121834	C	SORL1	Lambert (2013)	Lambert (2013)	0.039	-0.270	0.998	0.995	0.998
6	rs7593262	T	TREM2	Jonsson (2013),[45] Guerreiro (2013)[46]	Ruiz (2014)[47]	0.0016	0.889	0.762	0.726	0.668
7	rs1476679	T	ZCWPW1	Lambert (2013)	Lambert (2013)	0.287	0.078	0.995	0.996	0.995

Table 1. Genetic variants included in the genetic risk score. Ordering is by gene name assigned in the corresponding reference. Chr=Chromosome; Rsid=Reference SNP cluster ID; ALT-HRC=Alternative allele Haplotype Reference Consortium; MAF=minor allele frequency; R^2=imputation quality. The presented MAF for RS1 is representative of that in RS2 and RS3.

Dementia screening and surveillance

Participants were screened for dementia at baseline and subsequent centre visits using the Mini-Mental State Examination (MMSE) and the Geriatric Mental State Schedule (GMS) organic level.[24] Those with MMSE<26 or GMS>0 underwent further investigation and informant interview including the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX). Additionally, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study centre with medical records from general practitioners and the regional institute for outpatient mental healthcare. Available clinical neuroimaging data were reviewed when required for diagnosis of dementia subtype. A consensus panel headed by a consultant neurologist established the final diagnosis according to standard criteria for dementia (DSM-III-R), and Alzheimer's disease (NINCDS-ADRDA). Follow-up until 1st January 2015 was virtually complete (96.8% of potential person years). Within this period, participants were censored at date of dementia diagnosis, death, loss to follow-up, or 1st January 2015, whichever came first.

Other measurements

We assessed educational attainment (lower, further, or higher education), smoking status (never, former, or current), and use of antihypertensive or lipid-lowering medication at baseline by interview. Lipid levels were measured from fasting serum at baseline. Hyperlipidaemia was defined as LDL cholesterol >4.9 mmol/L (190 mg/dL), or use of lipid-lowering medication. Blood pressure was measured twice on the right arm with a random-zero sphygmomanometer. Hypertension was defined as elevated systolic or diastolic blood pressure (>140/90 mmHg) or use of antihypertensive medication. Body mass index was computed from measurements of height and weight (kg/m^2). A diagnosis of diabetes was based on the use of blood glucose-lowering medication or a fasting serum glucose ≥7.0 mmol/L.

Analysis

Analyses included all non-demented participants who provided data on family history at baseline. Missing data on non-genetic covariates (≤1.3%) were imputed using 5-fold multiple imputation, based on determinant, outcome and included covariates. Distribution of covariates was similar in the imputed and non-imputed dataset. We determined the association between parental family history of dementia and risk of dementia and Alzheimer's disease, using Cox proportional hazard models, and stratified results by paternal and maternal family history (or both), sex of proband, and mean age of proband at time of interview. We verified that choice of x-axis (age versus follow-up time) did not affect the results. Subsequently, we determined risk of dementia and Alzheimer's disease per decade increase in age at onset in parents. To account for potential misclassification of determinant

(i.e. parents deceased at young age, or developing dementia after interview) or outcome (i.e. participants who did not yet reach old age at end of follow-up), we performed sensitivity analyses excluding family history of parents who died prematurely (<65 years), excluding participants <70 years at baseline, and excluding non-demented participants censored before age 80.

Next, we compared characteristics of the subset of participants with MRI to those without MRI using age- and sex-adjusted analysis of covariance (ANCOVA) for continuous and logistic regression for dichotomous variables. We then determined the association between family history (overall and stratified by sex of affected parent and age at parental diagnosis) and (standardised values of) total brain parenchymal volume, hippocampal volume, cerebral perfusion, volume of white matter hyperintensities, presence of lacunar infarcts (yes vs. no), and cerebral microbleed count (classified as 0, 1, or ≥2). For continuous outcome variables these analyses were performed using linear regression; for categorical outcomes we used logistic and multinomial regression. Age at parental diagnosis was stratified at 80, as this approximates the mean age at diagnosis in the general population (illustrated by a mean age of 80.7 years at diagnosis for our participants, and 78.5 years at time of parental diagnosis).

All analyses were adjusted for age (at time of interview or MRI scan where appropriate) and sex, and additionally in a second model for level of education, smoking habits, history of hypertension, hyperlipidaemia, diabetes, and body mass index. To account for known genetic risk, in a third and fourth model we additionally adjusted for *APOE* genotype, and *APOE* genotype plus the genetic risk score for Alzheimer's disease, respectively. All imaging analyses were furthermore adjusted for total intracranial volume and interval between interview and MRI scan.

Analyses were done using IBM SPSS Statistics version 23.0 (IBM Corp, Armonk, NY, USA). Alpha-level was set at 0.05.

RESULTS

Of 2,233 eligible participants, 2,078 (93.1%) provided data on parental family history. Family history was positive for dementia in 407 (19.6%) persons. Mean age at diagnosis in affected parents was 78.5 years. Baseline characteristics of participants are presented in Table 2.

During a mean follow-up of 12.2 years, 142 participants developed dementia, of whom 105 (73.9%) had Alzheimer's disease. Mean age at diagnosis in participants was 80.7 years.

Characteristics	All participants (*N*=2078)	With MRI (*N* =1150)	Without MRI (*N*=928)
Age	64.1 ±7.5	62.0 ±5.5	66.7 ±8.8
Female sex	1142 (55.0)	614 (53.4)	528 (56.9)
Level of education			
Lower	1081 (52.7)	552 (48.9)	529 (57.3)
Further	603 (29.4)	349 (30.9)	254 (27.5)
Higher	369 (18.0)	228 (20.2)	141 (15.3)
Smoking history			
Former	1047 (50.6)	587 (51.4)	460 (49.6)
Current	388 (18.7)	204 (17.8)	184 (19.8)
Hypertension	1235 (59.5)	599 (52.1)	636 (68.5)
Diabetes	268 (12.9)	110 (9.6)	158 (17.0)
Body-mass index (kg/m^2)	27.2 ±4.0	26.9 ±3.6	27.5 ±4.5
Hyperlipidaemia	611 (29.4)	330 (28.7)	281 (30.3)
APOE genotype			
ε3/ε3	1174 (57.7)	663 (58.7)	511 (56.5)
ε2/ε2 or ε2/ε3	292 (14.4)	161 (14.3)	131 (14.5)
ε2/ε4, ε3/ε4, or ε4/ε4	568 (27.9)	305 (27.0)	263 (29.1)
Genetic risk score for Alzheimer's disease	-0.10 ±0.32	-0.09 ±0.32	-0.10 ±0.34
Family history of dementia	407 (19.6)	229 (19.9)	178 (19.2)
Paternal	116 (5.6)	65 (5.7)	51 (5.5)
Maternal	273 (13.1)	156 (13.6)	117 (12.6)
Both	18 (0.9)	8 (0.7)	10 (1.1)
Age at diagnosis in affected parent	78.5 ±8.3	79.2 ±7.5	77.5 ±9.1

Table 2. Baseline characteristics. Data are presented as frequency (%) for categorial, and mean±standard deviation for continuous variables.

Parental family history of dementia was associated with all-cause dementia and in particular Alzheimer's disease, which was only partly explained by known genetic variants (Table 3). These associations were similar for paternal and maternal family history of dementia (Table 3), and did not vary significantly by sex of proband (HR 1.82 [0.99-3.38] in men versus 1.43 [0.84-2.44] in women; *P*-value for interaction=0.44). Results were unaffected by excluding participants whose parents died at young age (before the age of 65: HR 1.95 [1.00-3.82]), and grossly similar for participants aged below and above the mean age of 64 years at time of interview (HR 2.45 [1.69-3.56] versus 1.93 [1.28-2.93]; *P*-value for interaction=0.36).

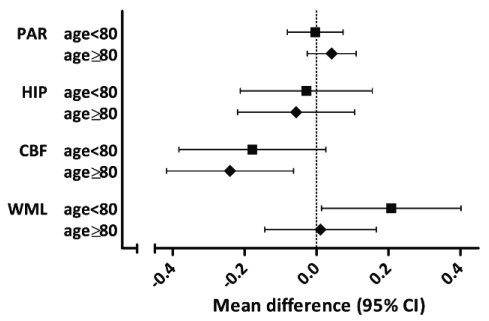
Associations between parental history of dementia and risk of dementia in probands were dependent on age at diagnosis in the parent (Table 3). Risk estimates gradually declined per advanced decade of age at diagnosis in parents, such that risk was highest when parents were diagnosed before age 80 (HR [95% CI] before: 2.58 [1.61-4.15] versus after 1.01 [0.58-1.77]). This trend was similar for Alzheimer's disease only. Accordingly, age at diagnosis in probands was highly correlated with age at diagnosis in their parents when parents were diagnosed before age 80 (*r*=0.57, *P*=0.001), but not thereafter (*r*=0.17, *P*=0.55). In sensitivity analyses to minimise potential information bias, age trends were similar when excluding

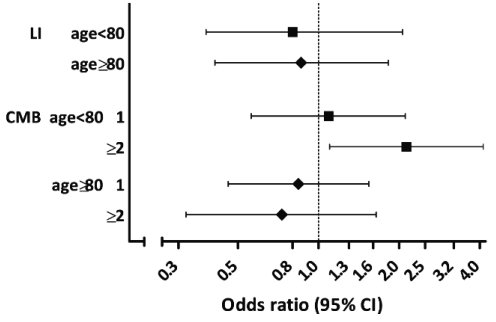
	Model I		Model II		Model III		Model IV	
	Dementia (n/N=142/2078) HR, 95% CI	**Alzheimer's (n/N=105/2078)** HR, 95% CI	**Dementia (n/N=142/2078)** HR, 95% CI	**Alzheimer's (n/N=105/2078)** HR, 95% CI	**Dementia (n/N=138/2034)** HR, 95% CI	**Alzheimer's (n/N=101/2034)** HR, 95% CI	**Dementia (n/N=122/1674)** HR, 95% CI	**Alzheimer's (n/N=88/1674)** HR, 95% CI
Family history of dementia	2.00, 1.40-2.85	2.37, 1.58-3.54	2.00, 1.40-2.86	2.37, 1.58-3.57	1.82, 1.26-2.63	2.12, 1.39-3.24	1.67, 1.12-2.48	2.01, 1.27-3.18
Paternal (n=116)	2.68, 1.58-4.55	3.19, 1.76-5.79	2.56, 1.51-4.35	3.00, 1.65-5.45	2.24, 1.30-3.88	2.62, 1.41-4.86	2.35, 1.31-4.22	2.68, 1.36-5.30
Maternal (n=273)	1.74, 1.13-2.68	2.02, 1.24-3.29	1.76, 1.14-2.72	2.07, 1.26-3.40	1.67, 1.07-2.62	1.94, 1.16-3.24	1.44, 0.89-2.34	1.75, 1.01-3.06
Both parents (n=18)	1.91, 0.47-7.79	2.78, 0.67-11.45	1.94, 0.46-8.14	2.73, 0.63-11.84	1.30, 0.30-5.64	1.62, 0.35-7.54	1.27, 0.30-5.48	1.81, 0.39-8.31

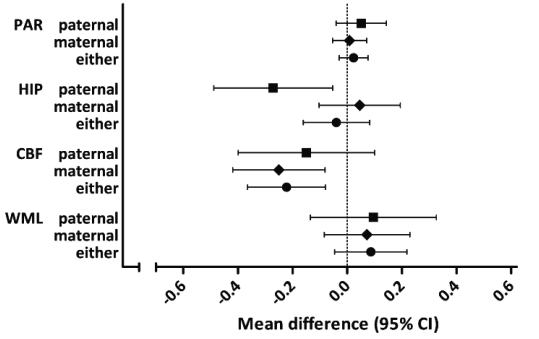
Table 3. Family history and risk of dementia. SD=standard deviation; HR=hazard ratio; CI=confidence interval; n=number of dementia cases; N=total sample size. Model I is adjusted for age and sex; model II additionally for educational attainment, hypertension, diabetes, hyperlipidaemia, smoking, and body-mass index; model III includes all variables from model II with additional adjustment for *APOE* genotype; and model IV additionally includes the genetic risk score for Alzheimer's disease.

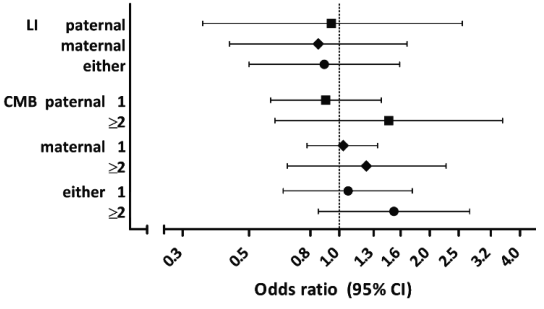
	n/N	**Model I** HR, 95% CI	**Model II** HR, 95% CI	**Model III** HR, 95% CI	**Model IV** HR, 95% CI
No parental family history	98/1573	REFERENCE	REFERENCE	REFERENCE	REFERENCE
Age at diagnosis in parent*					
<70	6/48	3.90, 1.69-8.98	3.93, 1.73-8.92	2.86, 1.18-6.94	2.71, 1.08-6.82
70-79	22/122	3.11, 1.95-4.94	3.09, 1.93-4.96	2.68, 1.65-4.36	2.54, 1.51-4.29
80-89	13/160	1.34, 0.75-2.39	1.35, 0.75-2.41	1.28, 0.73-2.24	0.97, 0.50-1.89
≥90	2/26	1.09, 0.27-4.41	1.08, 0.29-4.06	1.26, 0.31-5.05	1.25, 0.30-5.19
P-value for trend		<0.0001	<0.0001	<0.0001	0.001

Table 4. Family history of dementia and risk of dementia by age at diagnosis in parents. *Youngest age if both parents developed dementia. Model I is adjusted for age and sex; model II additionally for educational attainment, hypertension, diabetes, hyperlipidaemia, smoking, and body-mass index; model III includes all variables from model II with additional adjustment for *APOE* genotype; and model IV additionally includes the genetic risk score for Alzheimer's disease. HR=hazard ratio; CI=confidence interval; n=number of dementia cases; N=total sample size.









LEFT Figure 1. Family history and brain MRI. Imaging markers of neurodegeneration and small-vessel disease on MRI are presented for positive family history of dementia by age of parental diagnosis. PAR=parenchymal tissue volume; HIP=hippocampal volume; CBF=cerebral blood flow; WML= white matter lesions; LI=lacunar infarcts; CMB=cerebral microbleeds; OR=odds ratio; CI=confidence interval. The model is adjusted for age, sex, total intracranial volume, interval between interview and MRI, level of education, hypertension, diabetes, hyperlipidaemia, smoking, body-mass index, and *APOE* genotype.

RIGHT Figure 2. Maternal versus paternal predisposition for imaging abnormalities. Imaging markers of neurodegeneration and small-vessel disease on MRI are presented for paternal and maternal family history of dementia. PAR=parenchymal tissue volume; HIP=hippocampal volume; CBF=cerebral blood flow; WML= white matter lesions; LI=lacunar infarcts; CMB=cerebral microbleeds; OR=odds ratio; CI=confidence interval. The model is adjusted for age, sex, total intracranial volume, interval between interview and MRI, level of education, hypertension, diabetes, hyperlipidaemia, smoking, body-mass index, and *APOE* genotype.

non-demented participants censored before they reached age 80, or excluding participants <70 years at baseline (data not shown). Consistent with overall estimates in Table 2, known genetic risk factors accounted for only part of the large increased risk with parents affected before age 80.

Of all 2,078 participants who provided family history, 1150 (55.3%) underwent MRI, a median 5.6 years (IQR 5.1-10.6) after baseline interview. Compared to non-participants, MRI participants were generally younger, and had a more favourable cardiovascular risk profile (Table 2). Thirty-four participants who developed dementia between interview and MRI were excluded. Lacunar infarcts were seen in 95 (8.5%) individuals, and at least one cerebral microbleed in 251 (22.5%) individuals (1 in 144, and ≥2 in 107 individuals).

Overall, family history of dementia was not associated with total parenchymal volume or hippocampal volume, or with markers of small-vessel disease. However, after stratification for family history by age at parental diagnosis, we found that participants whose parents were affected at younger age had a larger burden of white matter lesions and cerebral microbleeds (Figure 1). In addition, those with positive family history had lower cerebral blood flow regardless of age at parental onset (Figure 1). Apart from smaller hippocampal volume with paternal family history, results again were similar for paternal and maternal family history (Figure 2), regardless of age at parental diagnosis.

DISCUSSION

In this prospective population-based study we found an increased risk of dementia with positive family history that is strongly dependent on parental age at diagnosis, but does not differ by paternal or maternal predisposition. Known genetic risk factors accounted for a relatively small share of parental risk. Remaining risk may in part be explained by observed associations of family history with cerebral hypoperfusion and increased burden of small-vessel disease in non-demented participants.

The excess risk of dementia with positive family history in our study is comparable with estimates from prior case-control studies.[25,26] The lack of attenuation after controlling for demographic and lifestyle factors supports family history as a measure of heredity rather than a marker of shared environmental factors. Moreover, known genetic risk factors explained only part of the association in our study, highlighting the important role of unidentified genetic factors involved in the aetiology of dementia.[27] Remaining risk may be due to unidentified epigenetic signatures, low-risk common variants, or high-risk rare

variants like ABCA7 and SORL1, but until these are identified, our findings support obtaining family history over genome testing (only).[28] The vast majority of familial excess risk was accounted for by parents diagnosed before age 80, in accordance with estimates modelled in a prior study.[29] Age at diagnosis correlated well among parents and probands in this group, with correlations similar to those among relatives with early-onset Alzheimer's disease.[30] Thus taking family history is much more informative when enquiring about parental onset of dementia, rather than dementia at any age. As a rule of thumb, 80 years seems a useful mark for differentiating risk in clinical practice, for preventive strategies, and for selection of participants for research purposes.

The increased risk of dementia with parental family history was paralleled by lower cerebral perfusion, and an increased burden of cerebral white matter hyperintensities and microbleeds when parents were affected at younger age. Although one other study did not find an association of white matter hyperintensities with family history,[8] loss of white matter integrity has been associated with family history in two smaller studies.[9,10] Moreover, cerebral hypoperfusion was associated with family history of dementia in one study,[16] and can also relate to reduced glucose metabolism reported with positive family history previously.[12-15] As hypoperfusion,[31,32] small-vessel disease,[33] and cerebral microbleeds[34] all carry an increased risk of dementia, these may reflect early pathophysiological changes in the brain of those predisposed for developing dementia. Subclinical changes in the brain occur up till decades before onset of dementia symptoms, and neuronal injury is thought to occur years before marked cerebral atrophy is seen on MRI.[35] This might explain why we did not observe an overall association between family history of dementia and total brain volume. Similarly, two other studies reported differences in white matter integrity, as well as amyloid-β42 and tau in cerebrospinal fluid, in the absence of volumetric brain differences.[10,36] As expected, the majority of dementia diagnoses in our study were of the Alzheimer subtype. Yet, these clinical diagnoses may partly reflect other pathology. In fact, mixed pathology is increasingly seen with dementia at higher age, and the risk conferred by a positive family history therefore likely reflects various aetiologies, of which we identified perfusion and small vessel disease as contributors.

Although several smaller studies have reported particular or exclusive associations of maternal compared to paternal family history of dementia with biomarkers of neurodegeneration,[6,12-16] other studies did not find such a difference.[5,7] In this population-based study, we did not find evidence of particular maternal transmission with either risk of dementia or imaging biomarkers. Of note, the majority of prior studies did not control for the effects of *APOE*, or even preferentially selected *APOE* ε4 carriers. As *APOE* may have a

more profound effect on risk of dementia in women,[3] this might account for part of the associations previously found with maternal family history.

Among the major strengths of our study are its population-based setting with detailed structured questionnaires, meticulous follow-up for dementia, and large sample of participants undergoing MRI. Yet, several limitations need to be discussed. First, albeit structured, interview questions remain susceptible to information bias, in particular regarding quantitative information such as age at diagnosis. Second, not all of our participants underwent MRI investigation and we cannot rule out selection bias with regard to the imaging analyses. Participants with MRI were generally younger with a favourable cardiovascular risk profile, but as they reported positive family history equally often as participants without MRI, this is unlikely to have affected relative risks. Third, despite similar results in sensitivity analyses, dementia onset after administrative censoring date in younger participants might have caused information bias. Fourth, part of the observed effect of family history may be attributable to identified high-risk rare genetic variants, such as ABCA7 and SORL1. We had no exome sequencing data available, but given the very low prevalence of yet identified variants these are unlikely to explain a large part of the observed effect. Fifth, although we adjusted for many risk factors that probands may share with their parents, some residual confounding with regard to socio-economic status may exist. Finally, the vast majority of participants in our study are of Caucasian descent, potentially limiting generalisability to other ethnicities.

In conclusion, enquiring age at parental diagnosis greatly enhances the value of taking a family history of dementia. Unexplained heredity is substantial, and may in part be attributed to cerebral hypoperfusion and small-vessel disease. Our findings do not support a preferential risk with maternal compared to paternal family history.

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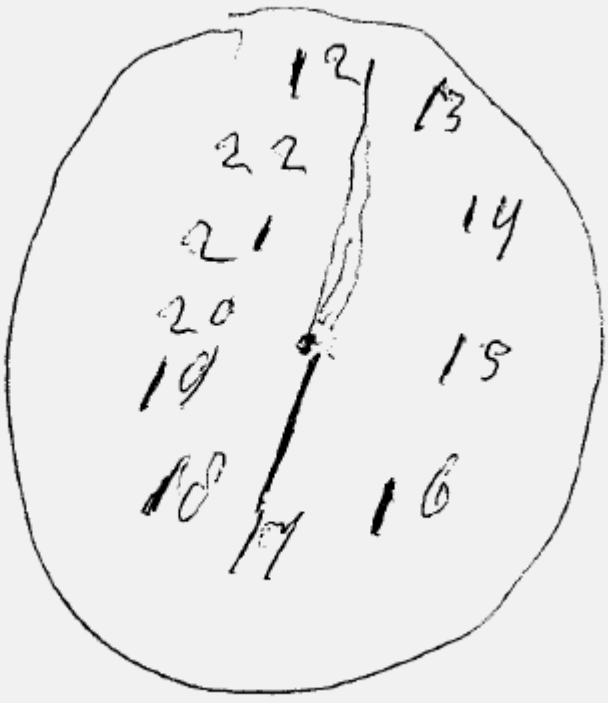
Chapter 5.4

Common genetic variants for risk prediction



ABSTRACT

Alzheimer's disease is the most common type of dementia, and one of the most heritable diseases in the elderly. Its major genetic determinant is the apolipoprotein E (*APOE*) gene, but twenty-three other common genetic variants have been identified which could be applied to risk stratification. We determined the effects of these twenty-three variants and *APOE* on cumulative incidence and age of onset of dementia between 1990 and 2016 in a prospective population-based cohort of 12,255 cognitively healthy participants aged ≥45 years (59% female). Risk curves were stratified by *APOE* genotype, tertiles of a weighted genetic risk score (GRS) of the twenty-three genetic variants, and parental family history of dementia. During 133,123 person-years of follow-up (median 11.0 years), 1,609 participants developed dementia, of whom 1,262 (78.4%) were classified as Alzheimer's disease, and 4,590 persons died of other causes. The GRS modified the risks of dementia and Alzheimer's disease, above and beyond *APOE* genotype, in particular for *APOE* ε44 carriers (*P*-value for interaction=0.04). In *APOE* ε44 carriers the difference in risk of dementia by age 85 between the high and low risk GRS tertile was 37.2% (27.0% for Alzheimer's disease), translating into a 7- to 10-year difference in age at onset. Comparing risk extremes, i.e. *APOE* ε22/23 carriers with a low GRS versus *APOE* ε44 carriers with a high GRS, the risk difference by age 85 was 70.3% for all-cause dementia (7.2% versus 77.5%, *P*<0.0001), and 58.6% for Alzheimer's disease (4.1% versus 62.7%, *P*<0.0001). This translates into an 18- to 23-year difference in age at onset of dementia, and 18-29 years difference for Alzheimer's disease. Incorporating parental family history further enhanced this difference for dementia to 83.8% (7.2% versus 91.0%, *P*<0.0001). In conclusion, common genetic variants with small individual effects jointly modify the risk of dementia substantially, in particular in *APOE* ε4 carriers. These findings highlight the potential of common variants in combination with family history and *APOE* for risk stratification in the general population.

INTRODUCTION

Dementia, with Alzheimer's disease as its most common form, is a highly multi-factorial disease with a considerable genetic component.[1] The strongest common genetic risk factor for dementia is the apolipoprotein E (*APOE*) gene,[2] which has a protective allele (ε2), and a risk allele (ε4), in addition to the most common reference allele (ε3).[3] Carriers of the *APOE* ε4 allele are at high risk of developing dementia, with absolute risk estimates from case-control studies surpassing 50% by the age of 85, compared to less than 10% at this age for non-carriers.[4,5] Because of this high risk, there is an increasing interest in preferentially including homozygote *APOE* ε4 carriers in trials during the pre-symptomatic phase of dementia, in order to reduce the necessary duration and size of these costly studies.[6,7] However, the clinical manifestation of dementia varies widely,[8] with age at onset ranging from midlife to the ninth decade of life even within homozygote *APOE* ε4 carriers.[4] Risk estimates using *APOE* alone therefore remain imprecise, with limited applicability in the population.

In addition to *APOE*, twenty-three other common genetic variants have been identified in the past decade that significantly modify risk of Alzheimer's disease.[9-20] Recently, it has been shown that combining the effects of these twenty-three variants results in a polygenic risk score that is not only associated with risk of Alzheimer's disease,[21-23] but also with neuropathological hallmarks of Alzheimer's disease,[24] conversion of mild cognitive impairment to dementia,[25-27] and the age at onset on dementia in both *APOE* ε4 carries and non-carriers.[24] However, these findings await validation in sufficiently powered community-based cohort studies,[4,24] taking into account competing risk of death to prevent overestimation of absolute risks.[28] Moreover, we have previously shown that parental family history of dementia captures much of the yet unaccounted heritability,[29] and incorporation of family history along with common variants thus seems essential to achieve the precise predictions of genetic risk.

In the present study, we yield over 25 years of data from a large community-based cohort to determine the aggregated effect of common genetic variants, by themselves and in conjunction with *APOE*, on the risk and age at onset of all-cause dementia and Alzheimer's disease.

METHODS

Study population

This study is embedded in the population-based Rotterdam Study, details of which have been described previously.[30] In brief, all residents of the Ommoord district in Rotterdam, the Netherlands, aged ≥55 years were invited to participate in the study in 1990. Of 10,215 invitees, 7,983 agreed to take part (response rate 78%). The study was expanded twice: once in 2000, including 3,011 participants (response rate 67%) who had turned 55, or moved into the study area, and a second time in 2006, thereby lowering the entry age to 45 years, and including an additional 3,932 participants (response rate 65%). At total of 14,926 participants thus take part in the study. Follow-up examinations at a dedicated study centre take place every 3 to 4 years. The present study includes all 12,255 initially non-demented participants who contributed follow-up time after the age of 60 years.

Screening and surveillance for dementia

Participants were screened for dementia at baseline and subsequent centre visits with the Mini-Mental State Examination and the Geriatric Mental State Schedule organic level.[31] Those with a Mini-Mental State Examination score <26 or Geriatric Mental State Schedule score >0 underwent further investigation and informant interview, including the Cambridge Examination for Mental Disorders of the Elderly. In addition, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study database with medical records from general practitioners and the regional institute for outpatient mental health care. This linkage allows detection of interval cases of dementia between centre visits. Available clinical neuroimaging was used when required for diagnosis of dementia subtype. A consensus panel led by a consultant neurologist established the final diagnosis according to standard criteria for dementia (DSM-III-R) and Alzheimer's disease (NINCDS–ADRDA).[32] Follow-up for dementia until 1st January 2014 (original cohort), 1st January 2015 (first expansion), and 1st January 2013 (second expansion) was near-complete (92% of potential person-years).

Genotyping and family history

DNA was extracted from blood samples drawn by venepuncture at the baseline visit, and genotyping done using commercially available arrays, with quality control separately per subcohort.[33] Preparation for imputation was done using scripts, which are provided online (HRC or 1000G Imputation preparation and checking: http://www.well.ox.ac.uk/~wrayner/tools/ version 4.2.1). Imputation to the Haplotype Reference Consortium (HRC) was facilitated by the Michigan Imputation server.[34] The server used SHAPEIT2 (v2.r790) to phase the data, and imputation to the HRC reference panel

(v1.0) was performed with Minimac 3. *APOE* genotype was determined using polymerase chain reaction on coded DNA samples for the initial cohort,[32] and with a bi-allelic TaqMan assay (rs7412 and rs429358) in the two cohort expansions. In 2.8% of individuals in the original cohort, 0.5% in the first expansion, and 4.6% in the second expansion in whom *APOE* genotype was not directly determined, it was imputed using '*best guess*' imputations (i.e. rounded dosages) of rs7412 (ε2 allele variant) and rs429358 (ε4 allele variant). Data of these imputations were concordant with direct genotyping for the ε2 and ε4 alleles in 98.9% and 98.2% of samples, respectively. In total, *APOE* genotype was available for 11,375 (92.8%) participants. Parental family history of dementia was assessed during baseline interview, and available for 8793 (71.7%) participants.

Genetic risk score (GRS) computation

We included the 23 genetic variants that showed genome-wide significant evidence of association with Alzheimer's disease to calculate a weighted GRS using reported effect estimates as weights.[11,14-16] If multiple studies reported the effects of a variant, the effect estimate from the largest study was used. A summary of the included variants, applied weights, and corresponding discovery studies, is available in Chapter 5.3. We included only genetic variants associated with Alzheimer's disease, as the number of participants with other types of dementia for which were genetic evidence is available was small: 51 diagnoses of dementia in individuals with Parkinson's disease, 14 participants with dementia with Lewy bodies, and 6 cases of frontotemporal dementia. The GRS was calculated as the sum of the products of SNP dosages of the 23 genetic variants (excluding *APOE*) and their respective weights. All 23 variants selected for the calculation of the GRS were well imputed (median imputation score (R^2)> 0.993). The formula to calculate the GRS along with two examples is provided in Table 1. We split the population into a high, middle, and low risk category by tertiles of the GRS; the GRS in the lowest tertile was <-0.325671, and for the highest tertile >0.050230. To minimise survival bias, these boundaries were determined by those entering the study before age 60.

Analysis

First, we compared baseline characteristics across *APOE* genotypes with *APOE* ε33 as the reference genotype, and across tertiles of the GRS with the lowest tertile as the reference, using t-tests for continuous measures and χ-squared tests for categorical measures.

Participants were censored at the date of dementia diagnosis, death, lost to follow-up, or the administrative censoring date, whichever came first. We calculated the cumulative incidence, henceforth risk, of all-cause dementia and Alzheimer's disease up to the age of 100 years using the 'etmCIF' function from the package 'etm' with R version 3.2.3.[35-37] In

Assigned-gene	Formula for the calculation of the GRS	Example 1 (low-risk tertile)		Example 2 (high-risk tertile)	
ABCA7	Number of G alleles (or dosage) of rs4147929 * -0.135	2*-0.135 =	-0.27	1.998*-0.135 =	-0.26973
BIN1	Number of T alleles (or dosage) of rs6733839 * 0.188	1*0.188 =	0.188	1*0.188 =	0.188
CASS4	Number of C alleles (or dosage) of rs7274581 * -0.139	0*-0.139 =	0	0*-0.139 =	0
CD2AP	Number of G alleles (or dosage) of rs10948363 * 0.098	1*0.098 =	0.098	1*0.098 =	0.098
CELF1	Number of C alleles (or dosage) of rs10838725 * 0.075	1*0.075 =	0.075	0*0.075 =	0
CLU	Number of T alleles (or dosage) of rs9331896 * 0.146	0*0.146 =	0	2*0.146 =	0.292
CR1	Number of G alleles (or dosage) of rs6656401 * -0.157	2*-0.157 =	-0.314	1.002*-0.157 =	-0.157314
ECHDC3	Number of G alleles (or dosage) of rs7920721 * -0.067	2*-0.067 =	-0.134	1*-0.067 =	-0.067
EPHA1	Number of A alleles (or dosage) of rs11771145 * -0.102	1*-0.102 =	-0.102	0*-0.102 =	0
FERMT2	Number of C alleles (or dosage) of rs17125944 * 0.122	0*0.122 =	0	0*0.122 =	0
HLA-DRB1/5	Number of A alleles (or dosage) of rs111418223 * -0.108	1.469*-0.108 =	-0.158652	1.544*-0.108 =	-0.166752
HS3ST1	Number of G alleles (or dosage) of rs13113697 * -0.067	2*-0.067 =	-0.134	0*-0.067 =	0
INPP5D	Number of T alleles (or dosage) of rs35349669 * 0.066	0.002*0.066 =	0.000132	1*0.066 =	0.066
KANSL1	Number of G alleles (or dosage) of rs118172952 * -0.151	0.733*-0.151 =	-0.110683	0.041*-0.151 =	-0.006191
MEF2C	Number of A alleles (or dosage) of rs190982 * 0.08	1.997*0.08 =	0.15976	1.007*0.08 =	0.08056
MS4A6A	Number of G alleles (or dosage) of rs983392 * -0.108	0.001*-0.108 =	-0.000108	1*-0.108 =	-0.108
NME8	Number of G alleles (or dosage) of rs2718058 * -0.07	1*-0.07 =	-0.07	0*-0.07 =	0
PICALM	Number of G alleles (or dosage) of rs10792832 * 0.13	1*0.13 =	0.13	2*0.13 =	0.26
PTK2B	Number of C alleles (or dosage) of rs28834970 * 0.096	1*0.096 =	0.096	1.999*0.096 =	0.191904
SLC24A4-RIN3	Number of T alleles (or dosage) of rs10498633 * -0.104	2*-0.104 =	-0.208	0*-0.104 =	0
SORL1	Number of C alleles (or dosage) of rs11218343 * -0.27	0*-0.27 =	0	0*-0.27 =	0
TREM2	Number of T alleles (or dosage) of rs75932628 * 0.889	0*0.889 =	0	0*0.889 =	0
ZCWPW1	Number of T alleles (or dosage) of rs1476679 * 0.078	0.002*0.078 =	0.000156	1.999*0.078 =	0.155922
	Genetic risk score (sum of the above)		**-0.754395**		**0.557399**

Table 1. Genetic risk score computation. Formulas for calculation are accompanied by two examples from the Rotterdam Study.

short, the function provides age-specific estimates with 95% confidence intervals of the cumulative incidence from a modification of the Kaplan–Meier estimator,[38] adapted for left truncation.[39] We accounted for mortality as competing event in every analysis, and additionally for dementia due to other causes than Alzheimer's disease as competing event in the estimations of Alzheimer's disease risk. Risks curves were similar for *APOE* ε22 and *APOE* ε23 carriers, and for *APOE* ε24 and *APOE* ε34 carriers, and these were therefore pooled into *APOE* ε22/23 and *APOE* ε24/34 in subsequent analyses. We stratified analyses by 1) *APOE* genotype, 2) tertiles of the GRS, 3) the combination of *APOE* genotypes and GRS, and 4) the combination of *APOE*, GRS, and positive family history in at least one parent. We calculated the differences between the risk estimates by age 85 years as previously described.[37] Interaction on the multiplicative scale between *APOE* genotype and the GRS, as well as the variant components of the GRS was tested using Cox proportional hazards and a Fine and Gray competing risk regression models, adjusting for main genetic effects, age at study entry, and sex.

RESULTS

Baseline characteristics of the 12,255 participants are presented in Table 2. During 133,123 person-years of follow-up (median 11.0 years), 1,609 participants developed dementia, of whom 1,262 (78.4%) had Alzheimer's disease, and 4,590 persons died of other causes. Overall, cumulative incidence (i.e. lifetime risk) of dementia by the age of 100 was 31.4% (95% confidence interval [CI] 30.1-32.8), whereas risk of Alzheimer's disease was 25.0% (23.8-26.3).

Effects of *APOE* genotype and common variants

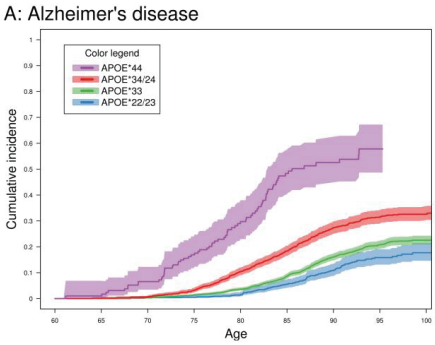
APOE genotype had a strong effect on risk of Alzheimer's disease (Figure 1A). By age 85 years, the risk for Alzheimer's disease was 48.3% (95% CI 40.1-57.3) for homozygous *APOE* ε4 carriers, and 18.4% (16.5-20.4) for heterozygous ε4 carriers. Risks were substantially lower in individuals without an ε4 allele: 8.6% (7.7-9.6) for homozygous ε3 carriers, and 5.5% (4.1-7.4) for ε22/23 carriers. Absolute risks were naturally higher for all-cause dementia, but with similar relative differences with respect to *APOE* genotype (Figure 1B). Stratified by tertiles of the GRS, the risk of Alzheimer's disease by age 85 was 8.1% higher in the highest tertile than in the lowest tertile (15.8% [14.1-17.6] versus 7.7% [6.5-9.1], $P<0.0001$) (Figure 2A). These differences were again similar for all-cause dementia (Figure 2B). Precise risk estimates with confidence limits for dementia are presented in Table 3.

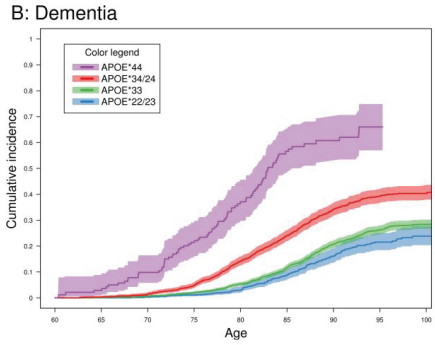
	All	Genetic Risk Score			*APOE* genotype					
		Low	Medium	High	ε44	ε34	ε24	ε33	ε23	ε22
	(*N*=12,255)	(*N*=3,402)	(*N*=3,292)	(*N*=3,317)	(*N*=261)	(*N*=2,608)	(*N*=312)	(*N*=6,662)	(*N*=1,453)	(*N*=79)
Incident Alzheimer's disease	1262 (10.3)	287 (8.4)	341 (10.4)	429 (12.9)	72 (27.6)	385 (14.8)	40 (12.8)	585 (8.8)	97 (6.7)	6 (7.6)
Incident dementia of other causes	347 (10.3)	88 (2.6)	90 (2.7)	116 (3.5)	11 (4.2)	95 (3.6)	13 (4.2)	162 (2.4)	38 (2.6)	3 (3.8)
Age (years)	67.48 (8.4)	67.2 (8.1)	67.0 (8)	67.0 (7.9)	64.8 (6)	66.6 (7.6)	67.3 (8)	67.3 (8.2)	67.2 (8.2)	69.2 (8.4)
Female sex	7164 (58.5)	1979 (58.2)	1922 (58.4)	1900 (57.3)	139 (53.3)	1518 (58.2)	171 (54.8)	3820 (57.3)	875 (60.2)	46 (58.2)
Educational attainment										
Primary	2210 (18.0)	606 (17.8)	589 (17.9)	566 (17.1)	37 (14.2)	471 (18.1)	44 (14.1)	1173 (17.6)	271 (18.7)	15 (19.0)
Further	8242 (67.3)	2319 (68.2)	2244 (68.2)	2291 (69.1)	179 (68.6)	1738 (66.6)	212 (67.9)	4562 (68.5)	991 (68.2)	54 (68.4)
Higher	1582 (12.9)	437 (12.8)	418 (12.7)	420 (12.7)	43 (16.5)	362 (13.9)	51 (16.3)	845 (12.7)	177 (12.2)	10 (12.7)
Smoking										
Never	4150 (33.9)	1157 (34)	1094 (33.2)	1121 (33.8)	70 (26.8)	830 (31.8)	96 (30.8)	2286 (34.3)	530 (36.5)	28 (35.4)
Former	5250 (42.8)	1470 (43.2)	1453 (44.1)	1423 (42.9)	126 (48.3)	1199 (46)	138 (44.2)	2850 (42.8)	592 (40.7)	32 (40.5)
Current	2479 (20.2)	684 (20.1)	665 (20.2)	696 (21.0)	60 (23.0)	512 (19.6)	69 (22.1)	1362 (20.4)	291 (20.0)	14 (17.7)
Systolic blood pressure (mmHg)	140 (22)	139 (22)	139 (21)	139 (22)	139 (23)	138 (21)	140 (23)	140 (22)	140 (22)	145 (25)
Diastolic blood pressure (mmHg)	77 (12)	77 (12)	77 (12)	77 (12)	78 (13)	76 (12)	77 (12)	77 (12)	77 (12)	78 (12)
Diabetes	1196 (9.8)	336 (9.9)	304 (9.2)	334 (10.1)	25 (9.6)	248 (9.5)	31 (9.9)	669 (10.0)	134 (9.2)	9 (11.4)
Body mass Index (kg/m^2)	26.8 (4)	26.9 (4)	26.7 (3.9)	26.9 (4.1)	26.6 (3.5)	26.6 (3.9)	26.8 (3.9)	26.8 (4.0)	27.1 (4.1)	27 (4.4)
Serum cholesterol (mmol/L)	6.2 (1.2)	6.3 (1.2)	6.2 (1.2)	6.2 (1.2)	6.4 (1.2)	6.4 (1.2)	6.1 (1.1)	6.2 (1.2)	5.9 (1.3)	6.1 (1.8)
Serum high density lipoprotein (mmol/L)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	1.3 (0.4)	1.3 (0.4)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)

Table 2. Baseline characteristics of the study population. Measurement details of non-genetic measures have been described in detail elsewhere.[31] Characteristics are presented as mean (standard deviation) for continuous variables, and absolute numbers (%) for nominal and ordinal variables. *APOE*=apolipoprotein E genotype.

		All			*APOE* ε44			*APOE* ε34/24			*APOE* ε33			*APOE* ε22/23		
GRS	**Age**	**Risk (95% CI)**	***N***	***P*-value**	**Risk (95% CI)**	***N***	***P*-value**	**Risk (95% CI)**	***N***	***P*-value**	**Risk (95% CI)**	***N***	***P*-value**	**Risk (95% CI)**	***N***	***P*-value**
All	*65*	*0.2% (0.1-0.4)*	*5220*		*2.9% (0.9-8.8)*	*136*		*0.3% (0.1-0.9)*	*1272*		*0.1% (0.0-0.3)*	*2874*		*0.0% (0.0-0.0)*	*678*	
Low		0.1% (0.0-0.5)	1466	REF.	0.0% (0.0-0.0)	40	REF.	0.0% (0.0-0.0)	349	REF.	0.2% (0.1-0.9)	871	REF.	0.0% (0.0-0.0)	205	REF.
Medium		0.1% (0.0-0.5)	1410	0.48	2.3% (0.3-15.0)	43	0.16	0.3% (0.0-1.9)	402	0.16	0.0% (0.0-0.0)	775	0.08	0.0% (0.0-0.0)	190	N/A
High		0.4% (0.2-0.9)	1462	0.05	7.5% (1.9-27.2)	34	0.07	0.5% (0.1-2.1)	377	0.08	0.1% (0.0-0.8)	855	0.28	0.0% (0.0-0.0)	195	N/A
All	*70*	*1.0% (0.8-1.3)*	*5670*		*9.8% (5.8-16.4)*	*128*		*1.4% (0.9-2.2)*	*1370*		*0.6% (0.4-1.0)*	*3170*		*0.3% (0.1-1.1)*	*743*	
Low		0.8% (0.5-1.4)	1584	REF.	0.0% (0.0-0.0)	45	REF.	0.5% (0.1-2.1)	384	REF.	1.2% (0.7-2.1)	941	REF.	0.0% (0.0-0.0)	214	REF.
Medium		0.8% (0.5-1.4)	1535	0.46	9.2% (3.6-22.7)	39	0.02	1.4% (0.6-3.2)	422	0.10	0.1% (0.0-0.8)	863	0.002	0.5% (0.1-3.4)	211	0.16
High		1.4% (0.9-2.2)	1633	0.06	22.4% (11.8-40.1)	27	0.0008	2.0% (1.0-3.9)	415	0.03	0.6% (0.3-1.4)	965	0.12	0.0% (0.0-0.0)	226	N/A
All	*75*	*3.1% (2.6-3.5)*	*5423*		*22.1% (16.2-29.8)*	*108*		*4.8% (3.8-6.0)*	*1326*		*2.0% (1.6-2.6)*	*3035*		*1.1% (0.6-2.1)*	*730*	
Low		2.1% (1.6-3.0)	1541	REF.	12.6% (5.8-25.9)	36	REF.	2.9% (1.7-5.0)	389	REF.	1.7% (1.1-2.8)	908	REF.	0.8% (0.2-3.3)	208	REF.
Medium		3.3% (2.5-4.2)	1517	0.02	20.5% (11.3-35.8)	30	0.15	5.7% (3.9-8.2)	400	0.02	1.6% (1.0-2.6)	867	0.43	1.8% (0.7-4.6)	220	0.19
High		3.9% (3.1-4.9)	1550	0.002	40.0% (26.4-57.3)	23	0.002	6.1% (4.2-8.7)	406	0.01	2.3% (1.5-3.4)	905	0.20	0.4% (0.1-2.8)	215	0.27
All	*80*	*8.1% (7.5-8.8)*	*4254*		*37.1% (29.7-45.7)*	*64*		*14.1% (12.5-15.9)*	*987*		*5.3% (4.6-6.1)*	*2458*		*3.1% (2.1-4.5)*	*573*	
Low		5.9% (4.9-7.1)	1256	REF.	22.5% (12.7-38.0)	24	REF.	9.5% (7.1-12.5)	316	REF.	4.5% (3.4-6.0)	754	REF.	1.6% (0.6-4.3)	162	REF.
Medium		7.7% (6.6-9.1)	1190	0.02	36.1% (23.9-52.0)	18	0.08	13.9% (11.0-17.4)	290	0.02	4.3% (3.2-5.8)	710	0.40	4.3% (2.3-7.8)	173	0.05
High		10.7% (9.4-12.3)	1191	<0.0001	58.1% (43.1-73.9)	12	0.0003	20.1% (16.8-24.0)	275	<0.0001	6.5% (5.1-8.1)	734	0.03	2.8% (1.4-5.8)	170	0.19
All	*85*	*15.6% (14.7-16.5)*	*2630*		*56.6% (48.2-65.2)*	*23*		*24.0% (21.9-26.2)*	*580*		*11.6% (10.6-12.8)*	*1500*		*8.8% (7.0-11.1)*	*387*	
Low		11.6% (10.2-13.2)	790	REF.	40.3% (26.6-57.7)	11	REF.	17.8% (14.5-21.7)	194	REF.	8.8% (7.2-10.8)	480	REF.	7.2% (4.5-11.5)	106	REF.
Medium		15.0% (13.3-16.8)	734	0.002	56.9% (42.6-72.1)	5	0.07	22.1% (18.5-26.3)	173	0.05	10.7% (8.8-12.9)	435	0.09	11.6% (8.0-16.5)	120	0.06
High		20.4% (18.6-22.4)	700	<0.0001	77.5% (63.1-89.3)	4	0.0002	33.4% (29.3-38.0)	156	<0.0001	14.9% (12.8-17.3)	429	<0.0001	9.2% (6.1-13.7)	112	0.22
All	*90*	*24.4% (23.3-25.6)*	*1161*		*62.0% (53.5-70.7)*	*10*		*34.2% (31.7-36.8)*	*220*		*20.7% (19.3-22.2)*	*648*		*16.2% (13.7-19.2)*	*183*	
Low		19.3% (17.4-21.4)	353	REF.	45.1% (30.1-63.3)	5	REF.	28.8% (24.6-33.5)	71	REF.	16.1% (13.8-18.7)	219	REF.	12.0% (8.2-17.2)	58	REF.
Medium		24.8% (22.7-27.1)	308	0.0001	66.0% (51.3-80.2)	4	0.03	33.2% (28.8-38.1)	67	0.09	20.2% (17.5-23.1)	186	0.02	20.0% (15.1-26.1)	52	0.01
High		30.3% (28.1-32.7)	287	<0.0001	77.5% (63.1-89.3)	1	0.002	43.1% (38.6-47.9)	63	<0.0001	25.7% (22.9-28.8)	173	<0.0001	16.7% (12.1-22.6)	52	0.09
All	*95*	*29.6% (28.3-30.9)*	*316*		*66.0% (57.0-74.9)*	*2*		*39.3% (36.7-42.1)*	*49*		*26.3% (24.6-28.0)*	*178*		*21.5% (18.5-25.0)*	*58*	
Low		25.2% (23.0-27.6)	97	REF.	51.4% (34.7-70.5)	1	REF.	34.5% (29.9-39.7)	11	REF.	22.5% (19.8-25.7)	67	REF.	14.4% (10.0-20.3)	18	REF.
Medium		29.4% (27.0-31.9)	86	0.008	75.2% (60.9-87.3)	1	0.02	37.3% (32.6-42.5)	20	0.22	24.6% (21.6-27.9)	49	0.18	26.1% (20.3-33.2)	18	0.003
High		36.4% (33.9-39.0)	70	<0.0001	77.5% (63.1-89.3)	0	0.01	49.3% (44.5-54.5)	11	<0.0001	31.4% (28.2-34.8)	44	<0.0001	24.7% (18.8-32.0)	16	0.007
All	*100*	*31.4% (30.1-32.8)*	*48*					*40.8% (38.0-43.7)*	*5*		*28.4% (26.6-30.2)*	*30*		*23.8% (20.3-27.8)*	*9*	
Low		26.2% (23.8-28.6)	16	REF.				34.5% (29.9-39.7)	2	REF.	24.1% (21.1-27.4)	11	REF.	14.4% (10.0-20.3)	3	REF.
Medium		32.0% (29.4-34.7)	13	0.0008				39.8% (34.6-45.5)	2	0.08	27.8% (24.4-31.5)	7	0.06	29.7% (23.2-37.6)	4	0.0003
High		38.3% (35.6-41.0)	10	<0.0001				50.3% (45.2-55.5)	2	<0.0001	33.7% (30.3-37.3)	7	<0.0001	27.2% (20.8-35.1)	2	0.002

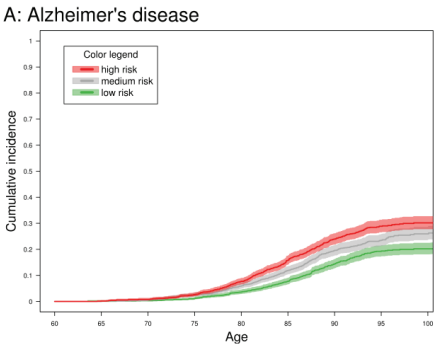
Table 3. Cumulative incidence of dementia by *APOE* genotype and the GRS. Empty cells imply no surviving (non-demented participants. The numbers of participants at risk within a group of GRS tertiles do not necessarily sum of the total at risk, because not all subjects with *APOE* status also had a known GRS profile. *APOE* = apolipoprotein E; GRS=genetic risk score; CI=confidence interval; N=number at risk; REF=reference; N/A=not applicable.

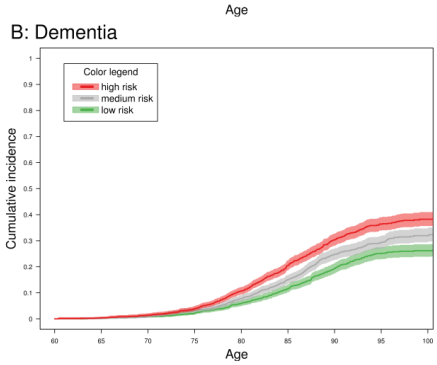




APOE*44	82	136	128	108	64	23	10	2	0
APOE*34/24	875	1272	1370	1326	987	580	220	49	5
APOE*33	1866	2874	3170	3035	2458	1500	648	178	30
APOE*22/23	437	678	743	730	573	387	183	58	9

LEFT: Figure 1. Risk curves of Alzheimer's disease (A) and dementia (B) by *APOE* genotypes. The risk curves show the cumulative incidence of Alzheimer's disease (A) and dementia (B). The shaded areas show the upper and lower 95% confidence limits of the corresponding cumulative incidence curve. The number of individuals at risk by age is shown under the graph.





high risk	954	1462	1633	1550	1191	700	287	70	10
medium risk	954	1410	1535	1517	1190	734	308	86	13
low risk	954	1466	1584	1541	1256	790	353	97	16

RIGHT: Figure 2. Risk curves of Alzheimer's disease (A) and dementia (B) by tertiles of the GRS. The risk curves show the cumulative incidence per 100 individuals of Alzheimer's disease (A) and dementia (B). The shaded areas show the upper and lower 95% confidence limits of the corresponding cumulative incidence curve. The number of individuals at risk by age is shown under the graph.

Effect of common variants on risk by *APOE* genotype

Risk estimates of dementia and Alzheimer's disease stratified by both *APOE* and the GRS groups are depicted in Table 3**.** A higher GRS was associated with increased risk within each of the separate *APOE* genotypes, but effects were largest and seen earliest in life for *APOE* ε4 carriers. This interaction between *APOE* and the GRS was significant for both risk of dementia (*P*=0.04), and Alzheimer's disease (*P*=0.03), and appeared attributable to various components of the GRS rather than one single variant (Table 4).

Assigned Gene	Rs-id	$P_{interaction}$
ABCA7	rs4147929	0.83
BIN1	rs6733839	0.83
CASS4	rs7274581	0.16
CD2AP	rs10948363	0.64
CELF1	rs10838725	0.37
CLU	rs9331896	0.08
CR1	rs6656401	0.09
ECHDC3	rs7920721	0.99
EPHA1	rs11771145	0.94
FERMT2	rs17125944	0.11
HLA-DRB1/5	rs111418223	0.01
HS3ST1	rs13113697	0.07
INPP5D	rs35349669	0.91
KANSL1	rs118172952	0.73
MEF2C	rs190982	0.18
MS4A6A	rs983392	0.44
NME8	rs2718058	0.41
PICALM	rs10792832	0.74
PTK2B	rs28834970	0.67
SLC24A4-RIN3	rs10498633	0.43
SORL1	rs11218343	0.80
TREM2	rs75932628	0.74
ZCWPW1	rs1476679	0.43

Table 4. Interaction of single variants in the GRS with *APOE* genotypes.

By age 85, the risk of Alzheimer's disease for homozygous ε4 carriers with a high GRS was 62.7% (47.2-78.2) compared to 35.7% (22.6-53.2) with a low GRS, corresponding to a risk difference of 27.0% (*P*=0.009). For heterozygous ε4 carriers, the risk difference by this age was 13.8% (*P*<0.0001), decreasing to 6.1% for homozygous *APOE* ε3 carriers (*P*<0.0001), and 0.7% for carriers of the ε2 allele (*P*=0.35). A similar trend was seen for dementia, with risk differences between a low and a high GRS by age 85 of 37.2% for homozygous *APOE* ε4 carriers (*P*=0.0002), lowering to 15.6% in heterozygous ε4 (*P*<0.0001), 6.1% in homozygous ε3 (*P*<0.0001), and 2.0% for ε2 carriers (*P*=0.22).

APOE ε2 carriers with a low GRS had the lowest risk by age 85 years of dementia (7.2% [4.5-11.5]), as well as Alzheimer's disease (4.1% [2.1-7.7]). The GRS did not discriminate much within the group of ε2 carriers before age 85, but was related to onset of dementia in the oldest old (Table 3). Homozygous carriers of the ε4 allele with a high GRS were at highest risk, reaching 77.5% (63.1-89.3) for dementia, and 62.7% (47.2-78.2) for Alzheimer's disease. Thus, between these genetic risk extremes there was 70.3% risk difference for dementia by age 85 (*P*<0.0001), and a 58.6% risk difference for Alzheimer's disease (*P*<0.0001).

Figure 3 illustrates the risk of dementia and Alzheimer's disease by age, *APOE* genotype and GRS, with increasing risk displayed in various colour gradients from green to red. This shows for example that homozygous *APOE* ε4 carriers with a high GRS attain 5% risk of dementia by age 64 (67 years for Alzheimer's disease), and 12.5% risk by age 67 (71 years for Alzheimer's disease). For comparison, *APOE* ε2 carriers with a low GRS attain 5% risk of dementia by age 82 years (85 years for Alzheimer's disease), and 12.5% by the age of 90 (100 years for Alzheimer's disease). This translates into a difference in age at onset in individuals with the highest versus the lowest genetic risk of 18-23 years for dementia, and 18-29 years for Alzheimer's disease. These differences in age at onset within *APOE* genotypes can also be appreciated in Figure 3. In homozygous *APOE* ε4 carriers a 40% risk of dementia is attained 9 years earlier by individuals with a high GRS (i.e. at 75 years) compared to those with a low GRS (i.e. 84 years). This was again similar for Alzheimer's disease (Figure 3).

Parental family history

Incorporation of parental family history of dementia further discriminated individuals at high risk from those at lower risk (Table 5). Estimates for all-cause dementia by age 85 went up to 91.0% (66.9-99.4) in the highest risk group (*APOE* ε44, high-risk GRS, and positive family history), increasing the absolute difference with the lowest risk group (*APOE* ε22/23, low-risk GRS, and no affected parent) to 83.8%. The *APOE* and GRS stratified risk estimates for all-cause dementia with and without a parental family history of dementia are shown in Table 5.

DISCUSSION

In this large population-based study, a GRS of common genetic variants modifies the risk and age at onset of dementia and Alzheimer's disease above and beyond the effect of *APOE*. The risk modification by the joint effect of common variants is most pronounced in *APOE* ε44 carriers in whom there is a difference of up to 10 years in age at onset between those with a low and high GRS. At the low-risk end of the spectrum, the same genetic variants in combination with the *APOE* ε22/23 genotypes identify a subgroup in the population that is at very low risk of dementia, with average age at onset of dementia nearly two decades later than individuals with the highest genetic risk. These differences can be further enhanced by incorporating parental history of dementia, implying future genetic discoveries may further benefit risk stratification.

The identification of subgroups at high genetic risk of dementia with an earlier onset in the general population has important implications for precision medicine. Pathological changes related to Alzheimer's disease begin to develop decades before the earliest clinical

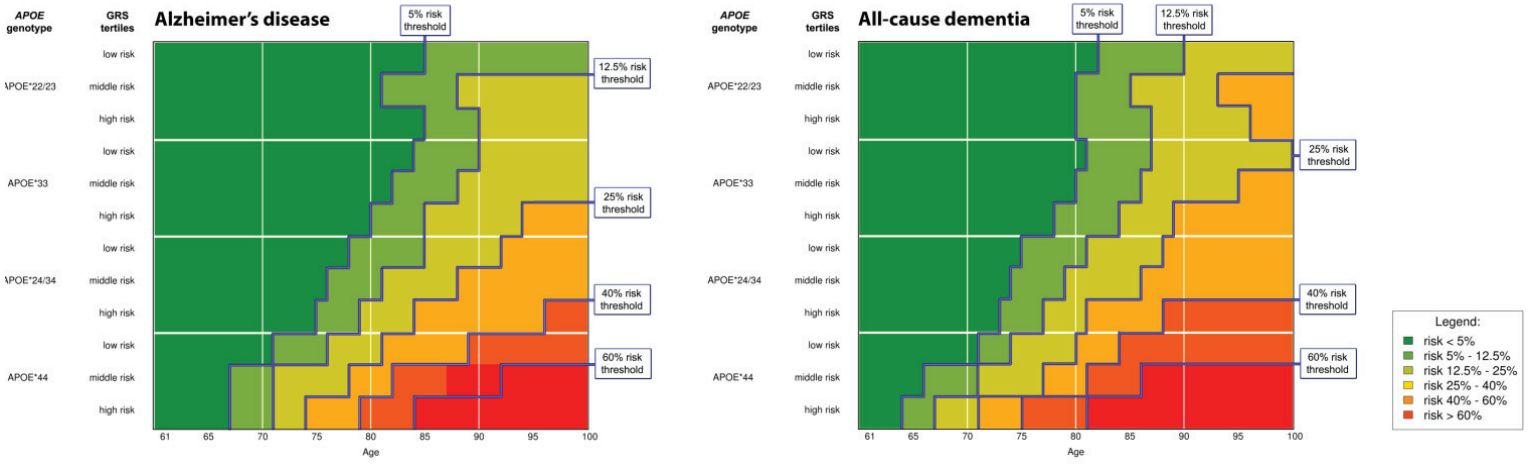


Figure 3. Risk of Alzheimer's disease (A) and dementia (B) by age (*APOE* genotype and GRS. The risk curves show the cumulative incidence per 100 individuals of Alzheimer's disease by age (*APOE* genotypes and tertiles of the GRS is shown. The risk is categorized and coloured in six risk categories (lower than 5% (between 5% and 12.5% (between 12.5%% and 25% (between 25% and 40% (between 40% and 60% and over 60% risk). The age by which the risks of 5% (12.5% (25% (40% and 60% is attained is marked with connected lines.

GRS	Age	*APOE* ε44			*APOE* ε34/24			*APOE* ε33			*APOE* ε22/23		
		FamHx– Risk (95% CI)	FamHx+ Risk (95% CI)	*N*	FamHx– Risk (95% CI)	FamHx+ Risk (95% CI)	*N*	FamHx– Risk (95% CI)	FamHx+ Risk (95% CI)	*N*	FamHx– Risk (95% CI)	FamHx+ Risk (95% CI)	*N*
Low	65	0.0% (0.0-0.0)	0.0% (0.0-0.0)	30	0.0% (0.0-0.0)	0.0% (0.0-0.0)	268	0.0% (0.0-0.0)	0.9% (0.1-6.2)	658	0.0% (0.0-0.0)	0.0% (0.0-0.0)	149
Medium	65	5.3% (0.8-31.9)	7.7% (1.1-43.4)	31	0.5% (0.1-3.7)	0.0% (0.0-0.0)	310	0.0% (0.0-0.0)	0.0% (0.0-0.0)	600	0.0% (0.0-0.0)	0.0% (0.0-0.0)	151
High	65	18.0% (4.5-57.7)	0.0% (0.0-0.0)	25	0.4% (0.1-3.1)	1.6% (0.2-11.1)	300	0.0% (0.0-0.0)	0.0% (0.0-0.0)	654	0.0% (0.0-0.0)	0.0% (0.0-0.0)	154
Low	70	0.0% (0.0-0.0)	0.0% (0.0-0.0)	39	0.9% (0.2-3.4)	0.0% (0.0-0.0)	353	0.7% (0.3-1.8)	1.5% (0.4-5.9)	855	0.0% (0.0-0.0)	0.0% (0.0-0.0)	192
Medium	70	9.6% (2.5-33.2)	14.3% (3.8-46.1)	35	1.5% (0.6-4.1)	1.0% (0.1-7.0)	388	0.1% (0.0-1.0)	0.0% (0.0-0.0)	800	0.0% (0.0-0.0)	3.7% (0.5-23.5)	197
High	70	33.0% (15.3-61.9)	20.0% (5.4-59.1)	25	1.9% (0.8-4.4)	3.6% (1.1-11.1)	389	0.7% (0.3-1.8)	0.5% (0.1-3.5)	870	0.0% (0.0-0.0)	0.0% (0.0-0.0)	197
Low	75	9.3% (3.1-26.1)	23.1% (8.1-55.8)	35	3.0% (1.6-5.7)	3.8% (1.4-9.7)	365	1.1% (0.6-2.2)	2.5% (0.9-6.7)	854	0.5% (0.1-3.7)	3.7% (0.5-23.5)	196
Medium	75	9.6% (2.5-33.2)	44.6% (24.5-71.2)	29	5.3% (3.2-8.5)	7.9% (4.2-14.7)	374	1.3% (0.7-2.4)	3.6% (1.6-7.9)	820	1.1% (0.3-4.2)	6.5% (1.6-23.8)	211
High	75	42.7% (24.0-67.7)	45.8% (22.3-77.4)	22	6.8% (4.5-10.2)	7.3% (3.6-14.5)	378	2.0% (1.2-3.3)	3.9% (2.0-7.6)	836	0.5% (0.1-3.5)	0.0% (0.0-0.0)	208
Low	80	18.9% (8.9-37.3)	23.1% (8.1-55.8)	25	8.7% (6.1-12.4)	13.3% (8.2-21.1)	298	3.6% (2.5-5.1)	6.7% (3.8-11.6)	711	1.0% (0.3-4.0)	3.7% (0.5-23.5)	156
Medium	80	36.4% (20.8-58.3)	44.6% (24.5-71.2)	19	14.7% (11.3-19.2)	12.5% (7.6-20.2)	267	4.0% (2.9-5.7)	6.2% (3.4-11.2)	671	2.6% (1.1-6.2)	12.7% (5.0-30.6)	167
High	80	60.2% (41.1-80.0)	62.8% (36.7-88.1)	12	18.9% (15.0-23.6)	26.8% (19.9-35.5)	255	5.7% (4.2-7.6)	9.5% (6.3-14.4)	683	3.0% (1.4-6.6)	0.0% (0.0-0.0)	165
Low	85	38.5% (23.1-59.4)	39.9% (15.7-78.0)	11	16.8% (13.1-21.5)	23.1% (16.2-32.3)	187	7.6% (5.9-9.7)	13.4% (9.0-19.7)	434	7.2% (4.2-12.0)	3.7% (0.5-23.5)	100
Medium	85	62.5% (43.8-81.1)	61.8% (39.6-84.0)	5	22.2% (18.0-27.3)	18.0% (11.8-26.8)	159	10.2% (8.2-12.6)	13.5% (8.8-20.2)	407	10.6% (6.9-16.1)	16.1% (7.0-34.6)	114
High	85	74.4% (56.4-89.3)	91.0% (66.9-99.5)	4	30.9% (26.0-36.5)	42.9% (34.8-52.1)	142	14.0% (11.7-16.8)	16.9% (12.3-23.0)	392	8.9% (5.5-14.1)	7.7% (2.6-22.2)	101
Low	90	46.1% (27.9-68.8)	39.9% (15.7-78.0)	4	26.6% (21.9-32.1)	42.3% (31.4-55.1)	66	15.1% (12.6-18.1)	20.1% (14.4-27.7)	196	10.1% (6.5-15.6)	21.3% (7.0-54.8)	53
Medium	90	62.5% (43.8-81.1)	74.9% (52.6-92.3)	4	33.0% (27.8-38.9)	32.4% (23.4-43.7)	63	20.1% (17.2-23.5)	18.5% (12.7-26.5)	171	19.5% (14.1-26.5)	23.8% (12.0-44.0)	47
High	90	74.4% (56.4-89.3)	91.0% (66.9-99.5)	2	41.9% (36.3-48.0)	49.3% (40.7-58.5)	59	24.2% (21.0-27.8)	27.4% (21.0-35.2)	158	15.7% (10.8-22.6)	20.8% (9.6-41.7)	43
Low	95	53.7% (33.8-76.2)		1	32.0% (26.8-37.9)	49.3% (36.9-63.3)	10	22.1% (18.9-25.8)	24.1% (17.5-32.8)	62	12.0% (7.8-18.1)	21.3% (7.0-54.8)	19
Medium	95				38.4% (32.7-44.6)	32.4% (23.4-43.7)	19	24.8% (21.5-28.5)	22.7% (15.4-32.8)	45	25.5% (19.0-33.6)	28.3% (15.1-49.2)	17
High	95				48.0% (42.1-54.4)	57.2% (48.0-66.7)	12	29.6% (26.1-33.6)	34.9% (26.9-44.3)	41	20.5% (14.5-28.6)	37.8% (21.6-60.6)	14

Table 5. Incorporation of family history. The cumulative incidence of dementia by *APOE* genotype, GRS, and parental family history of dementia. *APOE* = apolipoprotein E; GRS=genetic risk score; FamHx=parental family history; CI=confidence interval; N=number at risk; REF=reference; N/A=not applicable. Empty cells imply no surviving non-demented participants.

symptoms.[8] Preventive interventions therefore increasingly target asymptomatic individuals at younger age, but must preferentially selected individuals at high (genetic) risk of cognitive decline to render these costly trials feasible.[6,7,44] Selection of only high risk subgroups decreases the necessary sample size, and duration of trails,[6,24] although this should be weighed against the potential loss of generalisability of trial results. On the other end of the spectrum, individuals at extremely low risk of dementia might not want to risk trial exposure to treatment (side-effects). These persons are, however, of particular interest for inclusion in observational studies that aim to identify protective factors, or identify rare high-risk variants in individuals who do develop dementia against the odds.

The current study corroborates reports of variation in relative risks of common genetic variants by *APOE* genotype,[21,22,45] and adds that these differential effects extend to absolute risk and age at onset. Various biological pathways that have been implicated in Alzheimer's disease could be accountable for this genetic interaction.[46] Of suggested pathways involving endocytosis, haemostasis, cholesterol transport, hematopoietic cell lineage, protein folding, clathrin complexes, immune response, and protein ubiquitination,[46] *APOE* is a part of at least four.[46,47] Methodologically, a higher degree of misdiagnosis of Alzheimer's disease in ε4 non-carriers could also contribute to this interaction, but given the similar pattern for all-cause dementia, this seems less likely.

The overall estimates of the cumulative incidence of dementia and Alzheimer's disease in this study,[28,41] and the *APOE*-stratified risks by age 85 are comparable to previous reports that also accounted for competing risk.[41] The very similar patterns of risk curves for all-cause dementia and Alzheimer's disease were to be expected in view of the large share of dementia diagnoses comprised of Alzheimer's disease, but may also in part reflect effects of *APOE* and other genetic variants on other types of dementia and stroke.[47-50] Prior studies have suggested only marginal improvements of a GRS of common variants on discrimination between patients with Alzheimer's disease and controls,[21-23,25] but along with another recent study,[24] we show that effects are substantial for prospectively determined risk and age at onset. Although discrimination of the GRS may improve further by including increasing numbers of variants that have not been replicated, reported improvements in discrimination of such an approach have been marginal,[23] likely not outweighing additional costs.

Although we believe our results are valid, some limitations warrant mentioning. We estimated cumulative incidences up to a high age, including relatively many of the oldest old (e.g. 1,161 participants at the age of 90), but this could not prevent that stratification by *APOE* and the GRS tertiles left some subgroups with very small numbers at high age, rendering risk estimates less precise. Second, as the majority of Rotterdam Study

participants were native Dutch, results may not be fully applicable to other ethnicities. Third, refusal to participate in the study could have led to selection bias, most likely underestimating the absolute risk of dementia. Nevertheless, the initial response rate of the Rotterdam study is high (72%), compared to for example <10% in the UK Biobank, and the near-complete follow-up for dementia (92% of potential person-years) over prolonged follow-up of 26 years limits the impact of potential selection bias at baseline on absolute risk estimates. Fourth, family history of dementia provides more precise information for risk stratification if age at onset in relatives is taken into account,[29] but this information was not available for most of the participants in this study.

In conclusion, we show that the small effects of common genetic variants together significantly modify the risk of dementia, and determine a substantial part of the variability in age at onset. With the ever-expanding insight in the genetic make-up of Alzheimer's disease, these estimates will gain further precision, and will therefore require periodic updates in the future. Until then, our findings contribute towards better risk prediction of dementia, and may be used to improve efficacy of clinical trials.

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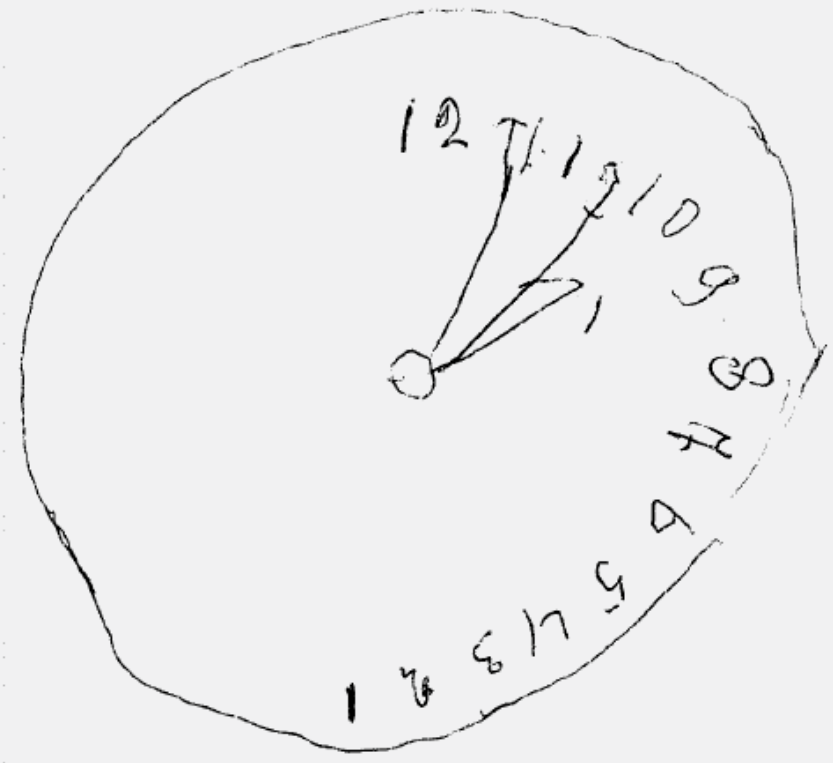
Chapter 5.5

Serum apolipoprotein E



ABSTRACT

APOE genotype is the most important genetic risk factor for dementia, and in particular Alzheimer's disease, and variation in gene expression may be reflected by differences in serum levels of apolipoprotein E (apoE). ApoE levels have consequently been suggested as potential biomarker for dementia, but its long-term association with risk of dementia or Alzheimer's disease is unknown. Between 1990 and 1993, we measured serum apoE by immunoassay in 1040 non-demented individuals (mean age 68 years; 59% women) from the population-based Rotterdam Study. We used Cox proportional hazard models to determine the risk of dementia and Alzheimer's disease (until 2014) in relation to apoE, adjusting for age, sex, educational attainment, cardiovascular risk factors, and additionally *APOE* genotype, and assessed additional predictive value using the integrated discrimination improvement (IDI) index. Serum apoE was strongly associated to *APOE* genotype (*P*-trend=1.0E-51, r^2=0.21). In men, apoE tended to be lower at higher ages, whereas in women the opposite was observed (*P*-trend=0.08 and 0.02, respectively). During a median follow-up of 15.7 years, 220 participants developed dementia, of whom 180 had Alzheimer's disease. Lower serum apoE was associated with an increased risk of dementia (HR [95%CI] per SD decrease: 1.32 [1.10-1.57]), and in particular Alzheimer's disease (HR 1.51 [1.23-1.86]), which remained statistically significant for Alzheimer's disease after additional adjustment for *APOE* genotype (HR 1.28 [1.00-1.62]). Associations were most profound in individuals heterozygous at the *APOE* locus (for all-cause dementia: HR 1.55 [1.25-1.90] versus 1.10 [0.84-1.43] with homozygosity; *P*-value for interaction=0.08). Serum apoE marginally improved 20-year prediction of Alzheimer's disease (IDI 0.007 [-0.002 to 0.023]), driven by a difference for heterozygous individuals (IDI 0.019 [0.0001 to 0.054]). In conclusion, serum apoE is associated with long-term risk of Alzheimer's disease in the general population, independent of *APOE* genotype, and might contribute to risk stratification as an easily accessible biomarker for Alzheimer's disease.

INTRODUCTION

Worldwide, approximately 48 million people are living with dementia, and this number is projected to nearly triple till 2050.[1,2] Although symptoms of dementia typically arise late in life, subclinical pathological changes in the brain occur up to decades before onset of symptoms.[3] Early identification of individuals at high risk of dementia is therefore essential to prevent manifestation of the disease. A reliable biomarker could aid in timely application of preventive strategies, selecting participants for neuroprotective trials, and disease monitoring. Various cerebrospinal fluid biomarkers have been assessed for these purposes in clinical populations, but plasma biomarkers that would allow long-term risk stratification in the general population are lacking.[3]

Apolipoprotein E genotype (*APOE*) is the major genetic risk factor for Alzheimer's disease, increasing lifetime risk for ε4 carriers 3 to 12-fold.[4] Various cross-sectional studies have shown that plasma levels of apolipoprotein E (apoE) are lower in patients with Alzheimer's disease,[5] and a recent Danish population-study found that lower levels of apoE are associated with increased risk of dementia and Alzheimer's disease.[6] However, median follow-up duration in the latter study was only four years, precluding any conclusion about long-term associations, which are most relevant for risk prediction. We aimed to determine the long-term association and predictive value of serum apoE for dementia and Alzheimer's disease in a population-based study.

METHODS

Study population

The current study was embedded within the population-based Rotterdam Study, details of which have been described previously.[7] In brief, the initial study population consisted of 7,983 individuals in the Ommoord district in Rotterdam, the Netherlands. Baseline examinations took place from 1990-1993. Of 7,152 participants who visited the research centre, we determined serum apoE in a random subset of 1,042 non-demented individuals. During the second follow-up visit from 1997-1999, measurements were repeated in a random subset of 338 of these individuals.

Measurement of serum apoE and *APOE* genotype

Blood samples were drawn by venipuncture from non-fasting subjects at baseline and from fasting subjects at follow-up, and samples were stored at -80°C. Serum apoE levels were measured by enzyme-linked immunosorbent assay (ELISA) at baseline, and plasma levels via

multiplex immunoassay on human multianalyte profiles (Myriad RBM Inc., Austin TX, USA; http://rbm.myriad.com) during follow-up. *APOE* genotype was determined using polymerase chain reaction on coded DNA samples, and classified into homozygous ε3 carriers, ε4 carriers (i.e. ε2/4, ε3/4, and ε4/4), and ε2 carriers (i.e. ε2/3 and ε2/2).

Dementia screening and surveillance

Participants were screened for dementia at baseline and subsequent centre visits using the Mini-Mental State Examination (MMSE) and the Geriatric Mental State Schedule (GMS) organic level.[8] Those with MMSE<26 or GMS>0 underwent further investigation and informant interview including the Cambridge Examination for Mental Disorders of the Elderly. Additionally, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study centre with medical records from general practitioners and the regional institute for outpatient mental healthcare. Available clinical neuroimaging data were reviewed when required for diagnosis of dementia subtype. A consensus panel headed by a consultant neurologist established the final diagnosis according to standard criteria for dementia (DSM-III-R), and Alzheimer's disease (NINCDS-ADRDA). Follow-up for dementia until 1st January 2014 was near-complete (93.9% of potential person years).

Other measurements

We assessed educational attainment, history of smoking (i.e. current, former, never) and use of antihypertensive or lipid-lowering medication at baseline by interview. Blood pressure was measured on the right arm with a random-zero sphygmomanometer. Non-fasting serum lipid levels were measured at baseline. Diabetes was defined as the use of blood glucose-lowering medication at baseline or a random serum glucose level ≥11.1mmol/L. Body mass index was computed from measurements of height and weight (kg/m^2).

Analysis

Analyses included all non-demented participants in whom serum apoE was determined. To guarantee model fit, serum apoE values of two individuals were recoded from +9 and +11 standard deviations (SD) from the mean to the third highest measurement of +4.3 SD. Missing covariate data (maximum 11.4%) were imputed using fivefold multiple imputation. We first determined the correlation of apoE with age, sex, and *APOE* genotype. We then determined the risk of dementia and Alzheimer's disease in relation to serum apoE levels, using Cox regression models. We tested for interaction on the multiplicative scale of apoE with age, sex, and heterozygosity at the *APOE* locus. We determined the predictive value of serum apoE over that of age, sex, and *APOE* genotype, expressed as changes in the area under the receiver operating characteristic curve (AUC) and integrated discrimination

improvement (IDI). In the subset of non-demented participants for whom we had a second consecutive apoE measurement, we determined the additive predictive value of this measurement for incident dementia, regarding the time in between first and second measurement as immortal person time. Analyses for prediction were done using R version 3.2.2 (packages 'risksetROC' and 'survIDINRI'). All other analyses were done using SPSS Statistics version 21.0 (IBM Corp, Armonk, NY, USA). Alpha (type 1 error) was set at 0.05.

RESULTS

Serum apoE was measured in 1,042 eligible individuals (mean±SD age 68.4±7.3, 59.3% women). Baseline characteristics of the study population are presented in Table 1. Serum apoE tended to lower with age in men, whereas in women the opposite was observed (*P*-trend=0.08 and 0.02, respectively; Figure 1A). Serum apoE was highest for the ε2/ε2, and lowest for the ε4/ε4 genotype (*P*-trend=$1.0*10^{-53}$; r^2=0.21; Figure 1B). Of study participants, 328 without dementia had apoE remeasured after on average 6.7 (SD 0.3) years. Correlation between the two subsequent measurements was moderate (Pearson's *r*=0.62; Figure 1C).

Characteristics	Study population
Age, years	68.4 ±7.3
Women	618 (59.3)
Educational attainment	
Lower	690 (66.6)
Further	282 (27.2)
Higher	64 (6.2)
Systolic blood pressure, mm Hg	136 ±20
Diastolic blood pressure, mm Hg	71 ±11
Antihypertensive medication	333 (32.0)
Diabetes	67 (7.3)
Serum cholesterol, mmol/L	6.7 ±1.2
Serum high density lipoprotein, mmol/L	1.3 ±0.4
Body mass index	26.6 ±3.8
Lipid-lowering medication	25 (2.4)
Smoking	
Former	433 (43.0)
Current	216 (21.4)
APOE genotype	
ε2/ε2	8 (0.8)
ε2/ε3	155 (15.0)
ε2/ε4	23 (2.2)
ε3/ε3	580 (56.0)
ε3/ε4	253 (24.4)
ε4/ε4	17 (1.6)
Serum apoE levels at baseline, mg/dL	2.86 ±1.49
Serum apoE levels at follow-up, mg/dL	4.62 ±2.23

Table 1. Baseline characteristics of the 1,042 participants. Values are presented as mean±standard deviation for continuous variables, and frequencies with percentages of total for nominal and ordinal variables.

During a median follow-up of 15.7 years (IQR 9.7-21.7) 220 individuals developed dementia, of whom 180 (81.8%) Alzheimer's disease. Lower serum apoE at baseline was associated with an increased risk of dementia, and in particular Alzheimer's disease (Table 1). These associations were attenuated, but remained statistically significant for Alzheimer's disease, after additional adjustment for *APOE* genotype (Table 2). Associations of serum apoE with incident dementia were stronger in those with a heterozygous compared to a homozygous genotype at the *APOE* locus (Table 3; *P*-value for interaction=0.08). There was no evidence of effect modification by age or sex (*P*≥0.73 for all-cause dementia). When stratifying analyses in 5-year time frames, risk estimates were similar throughout the study period (data not shown).

Overall, compared to a model with age, sex, and *APOE* genotype, adding serum apoE tended to marginally improve 20-year prediction of Alzheimer's disease (AUC 0.731 versus 0.726; IDI 0.007 (95% CI -0.002 to 0.023), *P*=0.093), but not all-cause dementia (AUC 0.718 versus 0.716; IDI 0.004 (-0.002 to 0.015), *P*=0.25). This was driven by an difference in individuals with a heterozygous *APOE* genotype (IDI 0.019 (0.0001 to 0.054), *P*=0.047; versus 0.001 (-0.003 to 0.014), *P*=0.48, for homozygous *APOE* genotype). Incorporation of repeated apoE measurements after 6.7 years did not improve prediction (data not shown).

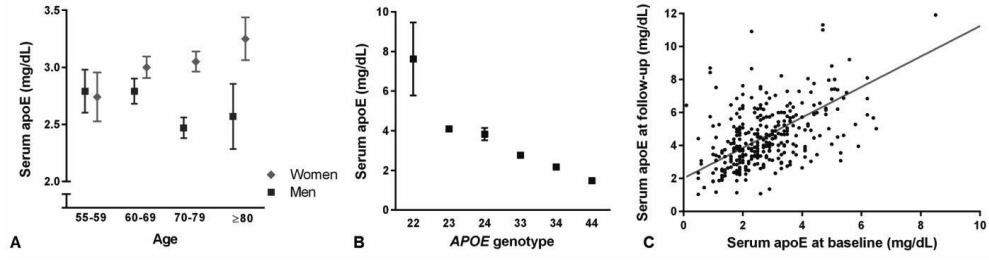


Figure 1. Serum apoE levels by age, *APOE* genotype, and correlation of repeated measures. Baseline serum levels of apoE are presented age- and sex-stratified (A), by *APOE* genotype (B), and in relation to a second measurement with a different immunoassay 7 years later (C). Values are depicted as group means with corresponding standard errors (A and B) and scatter plot of individual data points with regression line (C).

	N_{dem}/N_{total}	Model I HR, 95% CI	Model II HR, 95% CI	Model III HR, 95% CI	Model IV HR, 95% CI
Alzheimer's disease					
Highest tertile (≥3.2 mg/dL)	44/342	REFERENCE	REFERENCE	REFERENCE	REFERENCE
Middle tertile (2.2-3.1 mg/dL)	64/346	1.58, 1.08-2.33	1.37, 0.91-2.06	1.57, 1.06-2.33	1.37, 0.90-2.08
Lowest tertile (≤2.1 mg/dL)	72/354	2.18, 1.49-3.20	1.62, 1.06-2.47	2.15, 1.43-3.22	1.53, 0.97-2.42
Per SD decrease	180/1042	1.52, 1.25-1.84	1.31, 1.05-1.62	1.51, 1.23-1.86	1.28, 1.00-1.62
All-cause dementia					
Highest tertile (≥3.2 mg/dL)	61/342	REFERENCE	REFERENCE	REFERENCE	REFERENCE
Middle tertile (2.2-3.1 mg/dL)	77/346	1.37, 0.98-1.92	1.23, 0.86-1.77	1.36, 0.96-1.92	1.24, 0.86-1.79
Lowest tertile (≤2.1 mg/dL)	82/354	1.76, 1.25-2.47	1.37, 0.94-1.99	1.74, 1.21-2.50	1.33, 0.89-2.00
Per SD decrease	220/1042	1.32, 1.12-1.56	1.17, 0.97-1.41	1.32, 1.10-1.57	1.15, 0.93-1.41

Table 2. Serum apoE and dementia risk. Model I is adjusted for age and sex; model II for age, sex, and *APOE* genotype; model III for age, sex, educational attainment, and cardiovascular risk factors, with additional adjustment for *APOE* in model IV. SD=standard deviation; HR=hazard ratio; CI=confidence interval.

	Homozygote *APOE*			Heterozygote *APOE*		
	N_{dem}/N_{total}	Model I HR (95% CI)	Model II HR (95% CI)	N_{cases}/N_{total}	Model I HR (95% CI)	Model II HR (95% CI)
Alzheimer's disease						
Highest tertile	25/186	REFERENCE	REFERENCE	19/153	REFERENCE	REFERENCE
Middle tertile	33/226	0.88 (0.52-1.48)	0.83 (0.49-1.41)	31/118	3.17 (1.77-5.67)	2.36 (1.24-4.48)
Lowest tertile	30/193	1.25 (0.73-2.14)	1.06 (0.61-1.85)	42/160	3.89 (2.21-6.84)	2.66 (1.38-5.14)
Per SD decrease	88/605	1.34 (0.97-1.85)	1.23 (0.89-1.70)	92/431	1.68 (1.33-2.14)	1.41 (1.06-1.87)
All-cause dementia						
Highest tertile	32/186	REFERENCE	REFERENCE	29/153	REFERENCE	REFERENCE
Middle tertile	40/226	0.86 (0.54-1.37)	0.83 (0.52-1.34)	37/118	2.51 (1.53-4.12)	1.91 (1.10-3.32)
Lowest tertile	34/193	1.09 (0.67-1.77)	0.97 (0.59-1.60)	48/160	2.92 (1.80-4.74)	2.06 (1.17-3.65)
Per SD decrease	106/605	1.10 (0.84-1.43)	1.04 (0.80-1.34)	114/431	1.55 (1.25-1.90)	1.29 (1.01-1.66)

Table 3. Serum apoE and dementia risk by *APOE* genotype. Model I is adjusted for age and sex; model II for age, sex, and *APOE* ε4 carrier status. SD=standard deviation; HR=hazard ratio; CI=confidence interval.

DISCUSSION

In this population-based study, serum apoE levels were associated with risk of Alzheimer's disease, in particular in those with a heterozygous *APOE* genotype. Importantly, these associations were sustained up till 20 years of follow-up. Nevertheless, the added prognostic value of serum apoE over age, sex, education and *APOE* genotype was only marginal.

The mean levels of apoE in our study ranged from 2.9 mg/dL measured in serum of non-fasting subjects at baseline, to 4.6 mg/dL in plasma taken after fasting at follow-up nearly 7 years later. Correlation between measurements was high (r=0.62), but the levels at follow-up were thus higher on an absolute scale. Although this may be related to physiological processes, apoE levels are generally found somewhat higher in studies that measured plasma levels,[5,6] which may be explained by interactions between analytes and clotting factors or other additives.[5] Taking this into account, measured levels in our study were comparable to those obtained in other European and Asian studies,[5,6] albeit higher values have been reported for North-American populations.[5] To understand these differences and determine a reference standard for serum apoE levels, clear reporting of circumstances of blood withdrawal and methods of analyses in future studies is essential.

Within the central nervous system, apoE is produced mainly by astrocytes and plays an essential role in cholesterol transport and β-amyloid clearance.[9] In peripheral tissue, apoE is produced primarily by the liver and macrophages, and mediates lipoprotein metabolism.[9] ApoE in serum and CSF are thought to act independently, as animal work suggests only very limited transport of apoE (and other lipoproteins) across the blood-brain barrier in physiological conditions.[10,11] Moreover, phenotypes of *APOE* may differ between CSF and plasma,[12] and levels of apoE in CSF, but less so in serum, have been found to correlate with CSF levels of amyloid-β42.[12-14] The association between serum apoE and Alzheimer's disease, however, does suggest that peripheral apoE levels relate to pathology in the central nervous system. This is supported by similar correlations of *APOE* genotype with apoE levels in cerebrospinal fluid (CSF) and plasma in a large study,[13] albeit only with plasma levels in a smaller sample.[12] Upon direct comparison, correlation between serum and CSF apoE is low-moderate,[12-15] but possibly higher in patients with Alzheimer's disease than in healthy controls.[15] This might point to functional increases in response to pathology, or increased blood brain barrier permeability in patients with dementia,[16] allowing circulating serum apoE (with a relatively small molecular weight of 34kDa) to cross the blood-brain barrier into the central nervous system, and vice versa. At any rate, the profound associations of serum ApoE with incident dementia in heterozygous *APOE* carriers in our study suggest that

variation in gene expression, as previously demonstrated with *APOE* heterozygosity,[12] might be directly measured by peripheral levels of the gene product.

Risk estimates in our study were virtually unaffected by adjustment for cardiovascular risk factors. Although a few studies have reported modification of the effect of cardiovascular risk factors on dementia by *APOE* genotype,[17-19] no such studies are done with serum apoE, and our study was insufficiently powered to address this question. As associations of apoE with dementia remained substantial in effect size after accounting for *APOE* genotype, both may contribute in clinical risk stratification, especially in individuals heterozygous at the *APOE* locus. Nevertheless, improvements in prediction were only marginal, underlining the need for a combination of risk markers for an accurate prediction of Alzheimer's disease. Furthermore, although serum apoE seems to vary with age,[6] a second measurement of apoE nearly 7 years apart did not contribute to risk prediction in a subsample of our study. As correlations between repeated measures were high, this may indicate that any (pathophysiological) changes in serum apoE levels occur early in life and subsequently change proportionally in the absence of disease modifying intervention. It is likely that a combination of various serum biomarkers will be needed to improve prediction of Alzheimer's disease by blood tests, of which serum apoE might contribute in particular in those heterozygous at the *APOE* locus.

Although we believe our findings are valid, there are certain limitations to take into account. First, our sample size was relatively limited, which renders this study underpowered for associations with all-cause dementia. Of note, risk estimates were similar to those reported previously in the Danish population.[6] Second, as participants of the Rotterdam Study are predominantly Caucasian, our findings may not be applicable to other ethnicities. Third, the sensitivity of immunoassays for measuring different isoforms of apoE has been debated, as a previous mass spectrometry analysis did not show a correlation between serum apoE and Alzheimer's disease.[20] However, mass spectrometry reported correlations between apoE and genotype, as well as between apoE and sex, are in agreement with our findings.[20] Moreover, the correlation between different types of immunoassays was high, and the measurement error due to insensitivity of immunoassays would only be expected to dilute effect estimates.

In conclusion, serum apoE is independently associated with long-term risk of Alzheimer's disease, and may hold potential as an easily accessible biomarker for early detection of individuals at high risk of developing Alzheimer's disease. Nevertheless, excess predictive power in our study was limited, highlighting the need for development and concurrent use of additional serum biomarkers.

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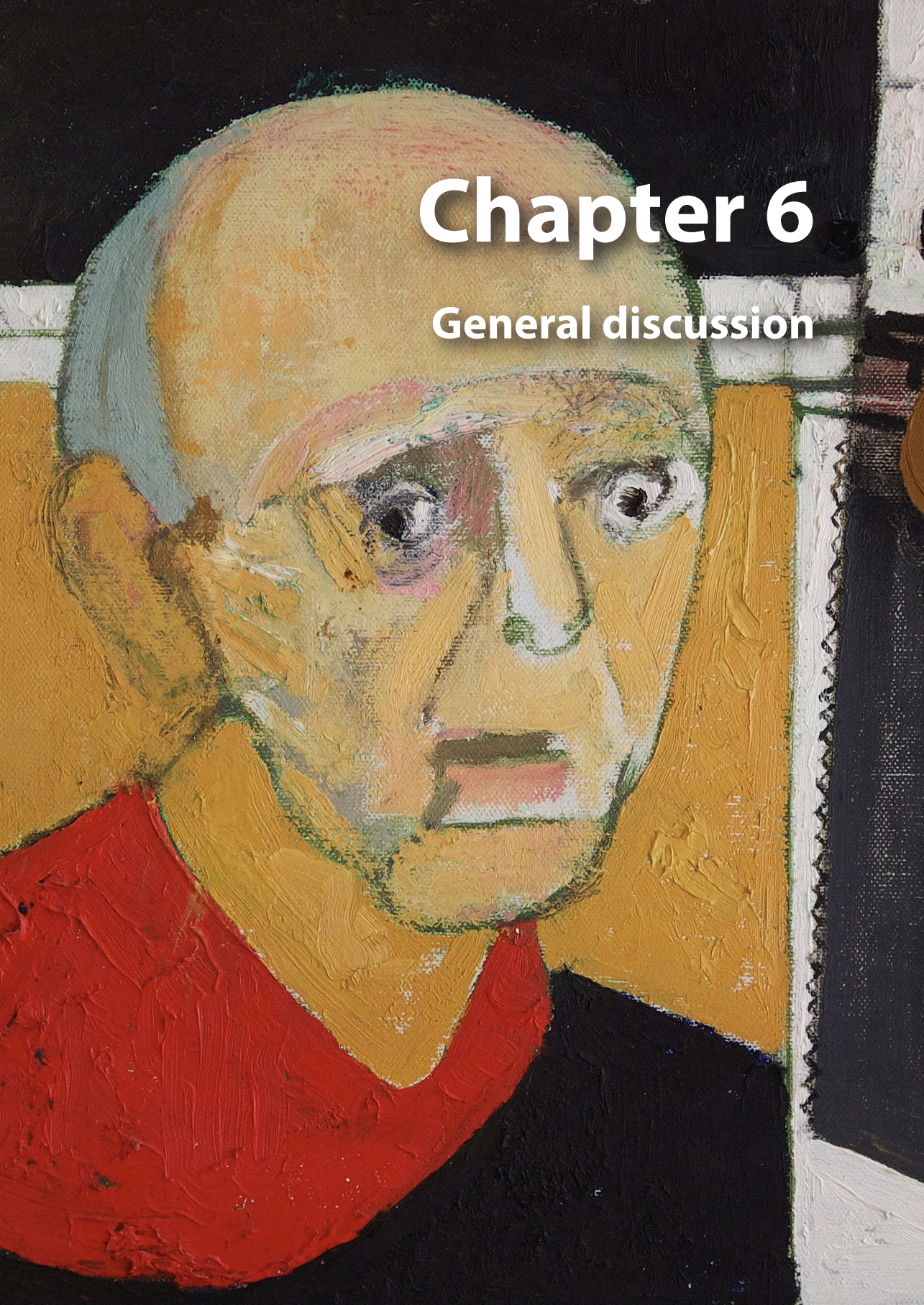

Chapter 6

General discussion

GENERAL DISCUSSION

The general discussion of the findings presented in this thesis marks the beginning of a journey's end. At this end, in all its presumed complexity, this dissertation is to compose but a few grains of sand; its value to be determined solely by that of the sand castle it may partly shape, and most importantly, the castle's ability to withstand the test of time. Let us hope that, like ancient Roman concrete, it will only gain in strength with repeated exposure to salty waves. In this final chapter, I will spout the first waves by interpreting the overall findings presented in this thesis in light of the larger body of published literature, addressing methodological vigour and imperfections, and outline the clinical and public health implications, as well as directions for future research.

FINDINGS IN PERSPECTIVE

During my medical studies, I was taught that the average systematic review of the literature yields about 1,500 citations for screening. Barely a decade later, the exponential growth in publications has boosted this number to some 4,000 published articles, exemplified by the findings described in Chapter 4.1. With nearly 200,000 articles about dementia in the PubMed library alone, one can find studies in support of virtually every possible hypothesis one can think of. It underlines the necessity, as well as the rising challenge of providing up-to-date literature reviews on a wide range of topics, in particular for observational studies, which are generally beyond the scope of Sir Iain Chalmers' 1993 Cochrane initiative. Given this abundance of available studies, the following disquisition should not be considered an exhaustive overview of the literature, as much as it is an overview of – subjectively – important studies in the field relating to this dissertation, combining convenient and (if such as thing exists) inconvenient truths on methodological merit more than anything else.

Dementia in numbers

One of the major criticisms about 'epidemiological research' is the impression that it sees merely noughts and ones where there is health and disease, failing to recognise the participants and patients behind the spreadsheet numbers. Somewhat ironically, one of the cornerstones of this dissertation consists of exactly that: numbers. The purpose, however, of presenting life expectancy, lifetime risks, and incidence rates is very much with the patient, or rather the wellbeing of the hitherto healthy individual, in mind. The message emerging from Chapter 2 is twofold. First, the burden of dementia is high, in particular among the very elderly, such that the effect of population ageing will surmount the decline in the age-

specific incidence of dementia observed in Europe and North America. Second, and I cannot stress this point enough: the key to curbing the dementia epidemic lies in prevention.

The first reports of a decline in the incidence of dementia came from Rochester (Minnesota, USA) and the Rotterdam Study,[1,2] and were based on observations in the late 1980s and 1990s. Yet, it took another five years with the publication of three further reports in 2016,[3-5] to spark cautious optimism regarding age-specific dementia risks in a larger audience. This development is somewhat reminiscent of the first report of a decline in mortality from coronary heart disease in 1964,[6] which received little attention until further confirmation in 1974 that indeed mortality rates had been declining since the early 1960s by about 20% within a decade.[7] If the four decades of quarrel about the causes that followed are any sign of what awaits the dementia field, quite a debate is at hand. But perhaps we can learn a few lessons from history. The incidence trends described in this thesis are in this sense a step towards consensus, as they corroborate the findings of individual studies using a consistent methodology in a set calendar period, and affirm that these trends have benefitted men and women equally. Moreover, they may provide a framework for further investigation of potential causes of these trends.

The main challenge in pinpointing causes of time trends is that there have been many concurrent changes, in public health, socioeconomic conditions, and medical treatment that may have contributed to changes in incidence rates (Figure 1). If history has thought us anything in this respect, it is the need for prolonged surveillance of disease and associated factors to enable modelling of trends and identification of causes.[8] Here dementia research has somewhat of a head start. Studies initiated with heart disease in mind, such as the Cardiovascular Health Study and the Atherosclerosis Risk in Communities study, already provide the infrastructure for dementia surveillance. This is an enormous advantage compared to the 1970s, and an important argument for continuing funding for disease monitoring in the population. Second, statistical and computational advances these days allow for easier and better modelling of trends than before. These benefits will be much needed to address outstanding questions about causes of trends, and their consequences on the expected burden of disease.

Despite the hopeful trends in dementia incidence described in this dissertation, there is still plenty of cause for concern. Recent contradictory reports from Japan,[12] China,[13] and Nigeria,[14] suggest that declines in the incidence may have been limited to Europe and North America. These observations temper any optimism about disease burden, in particular as the largest increases in dementia prevalence are expected to occur in Asia and Africa.[15] However, they may also create possibilities for identifying causes of trends by contrasting

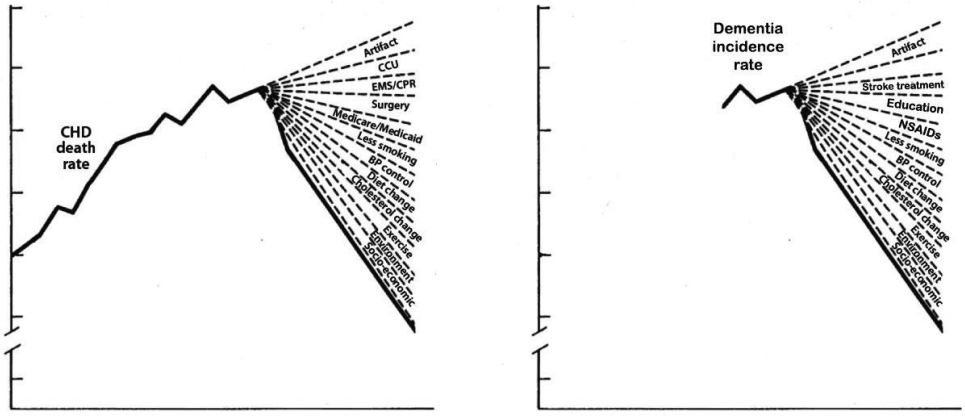

Figure 1. Identifying the causes of incidence trends. The left panel shows the initial thoughts about explanations for the trends in coronary heart disease, reproduced from Havlik and Feinleib,[9] whereas the right panel shows potential explanations for the presented trends in dementia incidence. Note that reliable observations of dementia incidence have only been available since the second half of 20th century. While there is considerable overlap, treatment factors – later held accountable for 40% of the CHD trend (versus 51% for preventive factors)[10,11] – are underrepresented for dementia, and education features prominently among the candidate preventive factors. On a historical note, the incidence trends described in this thesis were presented at a conference in Bethesda (Maryland, USA), dedicated to incidence trends in dementia, 39 years after the "Decline Conference" in Bethesda led to a consensus statement that incidence in heart disease mortality was in fact decreasing.[9]

observations between populations. This will require additional and continuous high-quality surveillance data not only from understudied areas, both from ongoing studies alike. Similar to heart disease,[8] we should caution that the rise of obesity,[16] diabetes,[17] and (on a global level) hypertension,[18] do not reverse trends in dementia over the coming decades. As eloquently put by physician historians David Jones and Jeremy Greene: "Even if death and taxes remain inevitable, cancer, coronary artery disease, and dementia may not. But cautious optimism should not become complacency. If we can elucidate the changes that have contributed to these improvements, perhaps we can extend them. Today, the dramatic reductions in coronary artery disease-related mortality are under threat. The incipient improvements in dementia are presumably even more fragile. The burden of disease, ever malleable, can easily relapse."[19]

A second cause for concern is the ageing population, as exemplified for the Netherlands in Figure 2. The incidence of dementia increases exponentially with age, very similar across European and North American populations, and seemingly without any flattening beyond the 9th decade of life.[20] The large, ongoing shift in population structure worldwide consequently leads to an increasing number of elderly individuals who are highly susceptible to dementia, but will the 20% decrease in incidence per decade, if sustained, be sufficient to

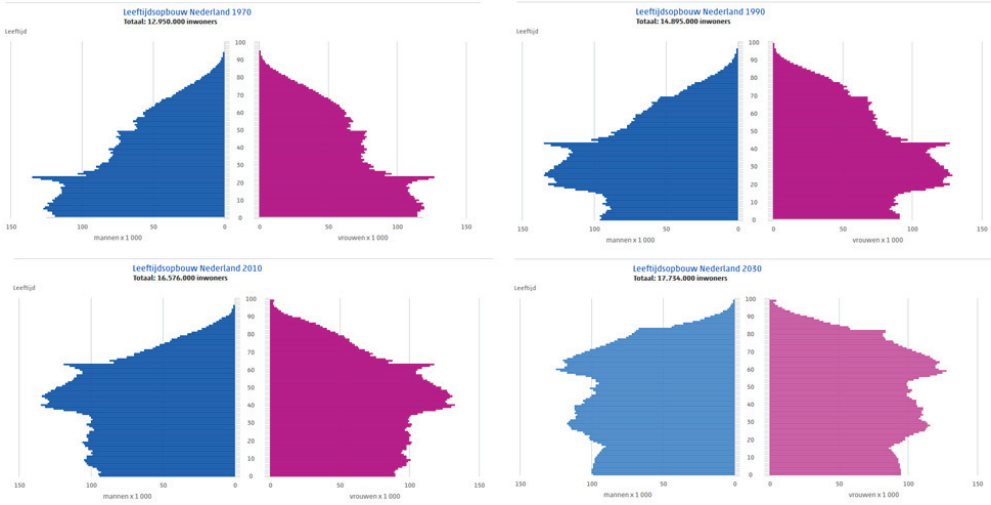

Figure 2. Change in population structure in the Netherlands from 1970 to 2030. Numbers are depicted in thousands, separately for men (blue) and women (pink). It is clearly visible how the traditional *pyramid* has eroded in a matter of decades. Data source: Central Bureau of Statistics in the Netherlands (CBS).

limit the lifetime risks and life years spent with dementia? This answer to this question is essentially determined by whether increases in life expectancy can be counterbalanced by reduced or postponed morbidity. Until the second half of the 20th century, the predominant view was that prolonged life expectancy would inescapably lead to higher burden of disease (Figure 3). But in 1980, internist James Fries proposed that this is not necessarily the case, as long as the factors accounting for prolonged survival are also linked with infirmity at old age.[21] This theory, designated the *compression of morbidity* would mean that longevity generally translates into a larger number of healthy life years (Figure 3). Fries later found support for his theory with a 35-year follow-up study among university alumni.[22] More recently, a comparative study of the first and second *Cognitive Function and Ageing Studies* in the UK found that the number of years lived with low and to a lesser extent high dependency has increased between 1991 and 2011.[23] However, these increases were substantially smaller than the concurrent increase in life expectancy in the UK,[24] suggesting that Fries' theory may hold at least in part. Nevertheless, causes of disability were not differentiated, and it may well be that in the absence of specific preventive interventions, the share of dementia in overall disability at old age in fact grows. Preventive efforts therefore remain indispensable, and as projected in this dissertation, are highly potent to reduce the burden of dementia by relatively minor postponements of its age at onset.

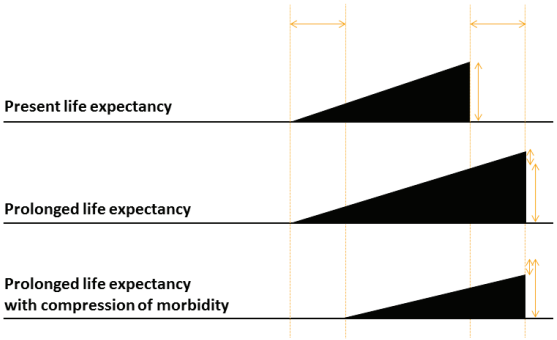

Figure 3. The burden of morbidity with changing life expectancy. The black triangle represents burden of disease across lifespan on the horizontal line. The arrows illustrate that prolonged life expectancy does not necessarily lead to increased burden of disease, as the same factors that extend lifespan also slow disease processes, and/or delay onset of disability.

The large modelled effects of relatively minor postponements in dementia onset are in line with earlier projections of hypothetical interventions on prevalence and incidence of dementia.[25] Such preventive efforts could be effective by either lowering the prevalence or impact of risk factors, or increasing cognitive reserve (Figure 4). Primary prevention (of shared risk factors for cardiovascular disease) is mostly directed at the former, whereas improvements in maternal health and education may be considered examples of the latter. Despite generally modest effect sizes at the individual level, these type of preventive intervention can greatly reduce the burden of disease at the population level.[26] It therefore pains to see that disregard of preventive medicine is widespread in research, with very little resources being dedicated to prevention.[27] Also for dementia, preventive interventions are likely to yield return of investment,[28] albeit the long preclinical disease course will require perseverance for some years from initiation of such interventions. Admittedly, targets for prevention of dementia are sparse when limiting oneself to available evidence from randomised controlled trials.[29] Following the failures of various dementia prevention trials in the late 1990s and early 2000s, trials have more frequently determined the effect of interventions on cognition as a more sensitive outcome measure than dementia. Although positive results have been subsequently seen in particular for trials assessing efficacy of physical activity and multi-domain interventions like the Scandinavian FINGER trial,[29,30] the recent French Multi-domain Alzheimer Prevention Trial (MAPT– testing similar interventions plus omega-3 supplements) found no significant benefit on cognitive decline over a 3-year period, and in an unselected population of elderly people in the Netherlands (the preDIVA trial), multi-domain vascular care intervention did not significantly lower dementia incidence.[31,32] These inconsistencies across trials employing closely aligned interventions emphasise that much work remains to be done to understand the specific pathways underlying their successes and failures. In contrast to intervention studies, there is ample

observational evidence for a role of modifiable risk factors in dementia, notably cardiovascular factors,[33] including the kind that is generally precluded from interventional study (e.g. effects of mid-life hypertension on disease in the elderly). In the absence of conclusive trial evidence – and more importantly without any signs of potential adverse effects – observational studies should in my view weigh heavily in recommending tight risk factor control for the prevention of dementia, as well as stroke and coronary heart disease. I believe it is important to advocate these treatments, as dementia is still too often seen as an inescapable consequence of ageing, with low awareness of modifiable risk factors to prevent cognitive decline (Figure 5). Such a reemployment of existing strategies can and should go hand in hand with the pursuit of better understanding of pathophysiological mechanisms.

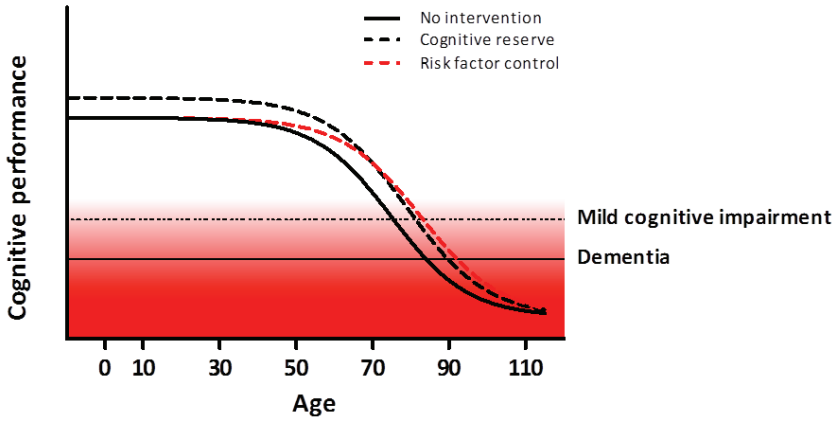

Figure 4. The potential of prevention. Targets for dementia prevention can address baseline cognitive ability (i.e. shift the curve upward by increasing *cognitive reserve*), or reduce exposure to risk factors such as hypertension (i.e. adjust the slope while shifting the curve right). Consequently, the threshold for functional impairment consistent with dementia will be reached at later age, potentially beyond the individual's lifespan.

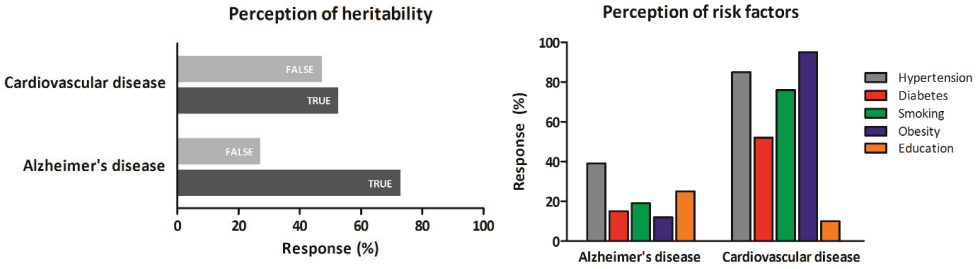

Figure 5. Perception of genetic influence and modifiable risk factors for Alzheimer's disease. Results from a survey among 174 community-dwelling respondents in Utrecht, the Netherlands. Participants were confronted with the proposition 'The risk of developing Alzheimer's disease and cardiovascular disease is for the most part genetically determined', and subsequently asked to identify risk factors for each disease among a list of the five presented risk factors and as many decoy answers (Ottink S, Van den Berg M & Wolters FJ, 2016).

Unravelling aetiology

Felix qui potuit rerum cognoscere causas. – Virgil, Georgics Book II.

Over the past decades, the field of dementia has moved from marking Alzheimer's disease as a distinct disease entity, to acknowledging that Alzheimer's disease is due to a multifaceted process that brings about pathophysiological changes in the brain along a seamless continuum, with its inception many years prior to clinical manifestation of the disease. Although Alzheimer's disease has traditionally been linked to amyloid pathology, its prevalence is high among individuals without dementia,[34] and correlations of amyloid with cognitive performance are generally low, both in humans and in purposefully designed mouse models.[35,36] It is often underappreciated that the vast majority of patients with dementia, among which many with clinical Alzheimer's disease, exhibit a multitude of pathologies upon autopsy.[37] Vascular pathology, including atherosclerosis, arteriolosclerosis, (micro)infarcts, and (micro)haemorrhages, is about as present as amyloid and tau pathology in the elderly, and the presence of vascular pathology is a capital determinant of the probability of having dementia or cognitive impairment with a given amyloid burden.[38] The recently reported association of late-life amyloid deposition in the brain with mid-life presence of cardiovascular risk factors in the population-based ARIC study further suggests that amyloid and vascular pathology should be seen in the context of another to understand the processes leading to clinical dementia.[39] Even for post-stroke 'vascular' dementia,[40] prolonged increases in risk after the acute event seem to indicate extensive underlying cerebrovascular pathology beyond initial infarct location and size, possibly of shared aetiology,[41] to explain a substantial part of this risk increase. Add to this the considerable overlap of amyloid and vascular pathology with α-synucleinopathies,[42,43] and it is hard to define the three quarters of dementia cases in the population that classify as clinical Alzheimer's disease as anything but *pars pro toto* for dementia. It illustrates above all the challenge to better disentangle phenotypes, which would be greatly facilitated by understanding of common and distinct pathways. In the following paragraphs, I will zoom in on the vascular component of dementia aetiology, guided by my study of cerebral haemodynamics and cardiovascular disease, and with special consideration for amyloid.

The term *autoregulation* in the cerebral circulation was coined by Niels Lassen in 1959,[44] who reviewed an "overwhelming body of knowledge" of over 350 papers published since it became possible to assess cerebral blood flow using the inert gas method (measuring arterial-venous gas difference) or the indicator dilution method (measuring the venous dilution of an intra-arterially injected indicator) 15 years prior. Until the 1930s, it was generally believed that cerebral blood flow and volume varied passively and within strict limits, based on the doctrine by Scottish physician Alexander Monro (1733-1817) and surgeon George Kellie (1770-1829) that an intracranial volume equilibrium must at all times

be maintained by changes in either cerebrospinal fluid or blood volume.[45] The possibility of a redistribution of blood within the cerebral vasculature, or by transference of cerebrospinal fluid was considered, but experimental data to support or refute the concept were unavailable till then. Fusing data from what was frankly a hodgepodge of studies, Lassen drew the no less accurate conclusion that cerebral blood flow remains constant over a wide range of blood pressure, and pinpointed in remarkable detail the autoregulatory mechanisms. An abstract view of his 'autoregulatory curve' is shown in Figure 6. Although the main focus of Lassen's work is on physiological control of cerebral blood flow, with mechanisms outlined in Chapter 1, he does briefly address 'various systemic disorders', among which there is a case of orthostatic hypotension, mention of cardiac and pulmonary diseases, and even a small paragraph on anaemia and polycythemia which brings to mind Chapter 3.5: "In anaemia and polycythemia the cerebral blood flow is increased and decreased respectively. […] The cerebral oxygen uptake has been found to be reduced in anaemia, but normal in polycythemia."[44] Although studies about 'organic dementia', 'senile psychosis', and 'cerebral arteriosclerosis' are then still rare, and generally comprising no more than a dozen patients, the hypotheses discussed are as topical today as they have ever been. It renders it all the more surprising that is has taken more than five decades since to present the first longitudinal studies about cerebral blood flow and some on the main flow regulating mechanisms in relation to risk of dementia.

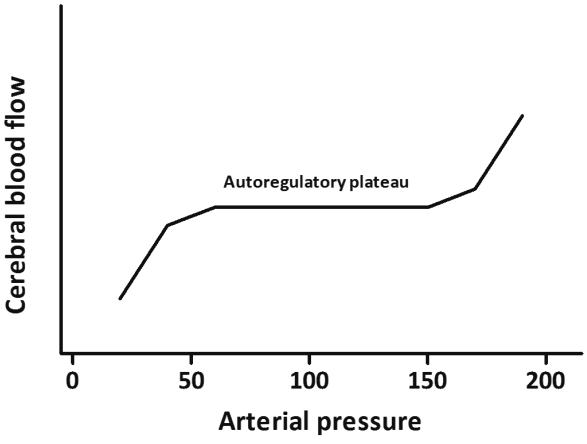

Figure 6A. The autoregulatory curve displays the range of blood pressure in which cerebral blood flow is held constant. Chronic hypertension can cause the curve to shift to the right.

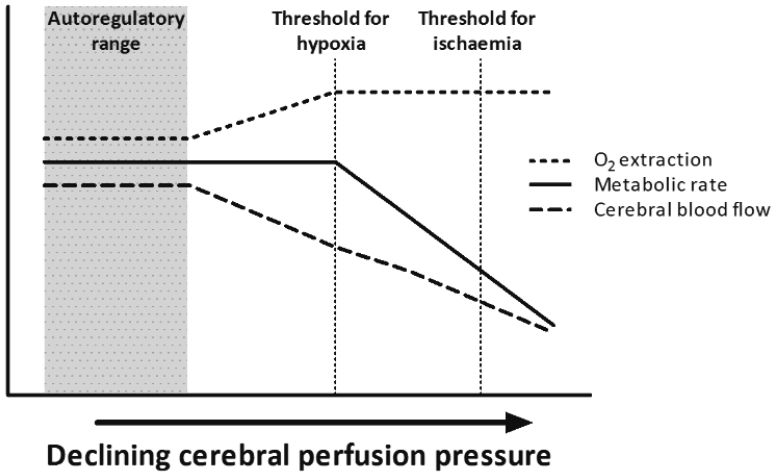

Figure 6B. Schematic overview of changes in metabolism with declining cerebral perfusion pressure. Protein synthesis gradually reduces from about 50% of its capacity with cerebral blood flow of 55mL/100mL/min to complete suppression at 35mL/100mL/min. With further lowering of perfusion electroencephalographic amplitudes start to decrease, and at about 15-20mL/100mL/min ATP breakdown is soon followed by anoxic depolarisation of cell membranes and disappearance of evoked potentials.[46]

Jointly, the studies described in Chapters 3 and 4 support a role of disturbed cerebral haemodynamics in the aetiology of dementia, whether brought on by cardiac (or autonomic) dysfunction, large artery disease, impaired neurovascular coupling, or disturbance in oxygenation. Until now, various studies had shown reduced cerebral blood flow in patients with Alzheimer's disease and mild cognitive impairment,[47-51] and correlations of amyloid-β with cerebral blood flow across the spectrum from cognitively healthy to demented,[52] but none had determined whether low cerebral blood flow precedes cognitive impairment. Interestingly, concurrent to the reporting of Chapter 3.1, it was shown in the Alzheimer's Disease Neuroimaging Initiative (ADNI) that increased cerebrovascular resistance exacerbates amyloidosis and predisposes to cognitive decline.[53] The applied method for estimation of resistance renders these results very similar to the interaction between blood flow and arterial pressure in Chapter 3.1. The fact that the associations in ADNI were independent of positron emission tomography defined neuronal metabolism, along with the associations over prolonged follow-up presented in this thesis, strengthen the notion that changes in cerebral haemodynamics may contribute to development of dementia. Nevertheless, the follow-up periods, up to 10 years in Chapter 3.1, are arguably insufficient to rule out reverse causation completely.[54] Whilst we await studies with repeated measurements of cerebral blood flow and cognition, extending over 10-15 years or even longer, alternative designs can teach us about the clinical and subclinical effects of changes in perfusion and transient or chronic hypoxia. A notable example of such a design is the multicentre Heart-Brain Study,[55] a longitudinal study of 645 participants, including 175

patients with vascular cognitive impairment, 175 with carotid occlusive disease, 175 with heart failure, and 120 control subjects. Aiming to unravel the haemodynamic link between cardiac dysfunction, brain pathology, and cognitive decline, all study participants undergo standardised neuropsychological testing, blood sampling, cardiac, vascular and brain MRI, and (in subsets of participants) cerebrospinal fluid sampling in a multidisciplinary setting. Joint efforts like the Heart-Brain Study, systematically covering multiple organ systems in clinical setting, may well prove an important step forward to understanding the effects of hypoperfusion on the brain, and integrating clinical care for patients in an organ transcending approach. In the coming paragraphs, I shall further discuss from various angles the study of cerebral haemodynamics. Guided by the mechanisms described in Chapter 1 and results presented in Chapter 3 and 4, I shall consecutively discuss flow regulatory mechanisms, disease related to reduced blood flow and oxygenation, and briefly touch upon effect modifying and mediating factors.

There is a substantial body of evidence to support that flow-regulating mechanisms are impaired in patients with dementia. Cerebrovascular reactivity,[56] as well as various measures of autonomic dysfunction are notoriously low in patients with dementia, in particular those with Parkinson's disease dementia and dementia with Lewy bodies.[57] Yet, it remains unknown at what time during the long preclinical disease course these functions become abnormal, as longitudinal studies about their change in time, in particular relating to dementia, are sparse. The results I describe in Chapter 3.3 are the first longitudinal evidence linking impaired vascular reactivity to development of dementia in the general population. Other studies reporting impaired vascular reactivity in healthy young *APOE* ε4 carriers,[58] and asymptomatic individuals with hereditary cerebral amyloid angiopathy support a role of vascular reactivity early in the disease process.[59] Patients with cerebral amyloid angiopathy are at high risk of cognitive decline,[60] and cortical atrophy in individuals with hereditary disease has been shown mostly mediated by vascular dysfunction.[61] With regard to autonomic dysfunction, three prospective cohort studies now show increased risks of dementia for orthostatic hypotension, with follow-up ranging from 6 to 25 years.[62,63] Of other markers of autonomic dysfunction, day-to-day blood pressure variability has recently been implicated in dementia risk,[64,65] while both blood pressure variability,[66,67] and heart rate variability[68] have been linked to cognitive decline. Various other markers, however, remain under-investigated. When Ewing and colleagues described their battery of cardiovascular autonomic function tests in the late 1970s,[69] this offered some guidance to clinicians as to the value of different tests, and their change over time.[70] The incorporated Valsalva manoeuvre, heart rate response to standing (30:15 ratio) and to deep breathing, and blood pressure response to standing and to sustained handgrip, are still often used, but few studies combine their measures, let alone other autonomic function tests, to assess

their joint and independent association with cognition. Similarly, measures of autonomic function are rarely combined with vascular reactivity to capture autoregulatory capacity in its totality. Although the complexity of human physiology often precludes proper investigation of more than one determinant at a time, the complexity of human physiology at times warrants investigation of more than one determinant at a time. In my view, cerebral haemodynamics are an emblematic example of this. The interactions with cerebral small vessel disease in Chapter 3.1, and between orthostatic blood pressure and heart rate response in Chapter 3.2 lend support to the idea that consequences of faltering mechanisms often occur only when multiple links in the chain fail simultaneously. This is illustrated furthermore by the link between exhaustion of cerebrovascular reactivity in the presence of severe carotid artery stenosis,[71] and subsequent risk of stroke and cognitive decline,[72-74] concomitant reduction of cerebral blood flow and vascular reactivity in patients with heart failure,[75] and a particular tendency to syncope in patients with orthostatic hypotension who also have impaired vascular reactivity.[76] Such findings also suggest that, even if decline in autonomic function is secondary to neurodegenerative pathology, it might still amplify pathology in the years preceding symptom onset, contributing to the generally exponential demise in trajectories of cognitive performance and brain imaging markers with ageing.

Apart from the common physiological challenges on cerebral autoregulation, various diseases may put them particularly to the test. Pulmonary disease, although no topic of intimate consideration in this thesis, is increasingly implicated as a risk factor for cognitive impairment and dementia.[81] Associations of chronic obstructive pulmonary disease and low arterial oxygen saturation with cerebral white matter hyperintensities are suggestive of hypoxic effects,[82] in addition to joint effects of smoking, systematic inflammation, and vasculopathy.[81] The theoretical importance of oxygenation (Figure 6) could furthermore be reflected in the associations of anaemia with risk of dementia, as described in Chapter 3.5. Although anaemia is notoriously associated with chronic disease, and further study into iron related mechanisms is certainly warranted,[83] I have illustrated that only substantial confounding, of an unlikely magnitude, would suffice to explain the observed effects by bias. The effects of prevention and treatment of anaemia on brain health remains to be tested in intervention studies, but could potentially mean that oxygenation is readily amendable to a meaningful level. Meanwhile, studies of physiological effects could be refined with the combination of haemoglobin levels, arterial, and venous oxygen content (equalling the oxygen bound to haemoglobin plus what is dissolved in blood: $1.34 * [Hb] * SaO_2 + (0.0031 * PaO_2)$), or oxygen extraction (estimated using Fick's equation, $oxygen\ consumption = blood\ flow * arteriovenous\ oxygen\ difference$). Unfortunately, arterial blood samples were not available in the studies yielded in this thesis, and oxygen saturation in only a small subset of participants.

Box 1. The inability to measure intracranial pressure non-invasively has made that much of current insight about brain perfusion originates from patients in need of invasive intracranial pressure monitoring. Several non-invasive measurement tools have been proposed to facilitate research about haemodynamic (patho)physiology, including the optic nerve sheet diameter (ONSD), blood flow velocities in the extracranial and intracranial ophthalmic artery (OA), and the combination of arterial pressure and pulsatility index on transcranial Doppler (TCD).[77] Correlation with invasively measured intracranial pressure in published studies was generally highest for OA (r=0.74-0.81), followed by ONSD (r=0.41-0.74), and TCD (r=0.31-0.94).[77] The optic nerve, as part of the central nervous system, is surrounded by subarachnoid space, and the intra-orbital optic nerve sheet displays elasticity with changes in intracranial (and consequently cerebrospinal fluid) pressure.[78] As the ONSD can be obtained from routinely acquired MR images, together with Dr. H.H. Adams, I used combined T1- and T2-weighted images (in the absence of a fat-suppressed T2-weighted sequence) to measure the ONSD (Figure 7). Because of natural variation in the optic nerve diameter (OND), correlating with sheet diameter, we measured both OND and ONSD, and calculated their ratio. Interrater agreement was moderate to good in the retrobulbar segment (at 3mm), for which we found a moderate negative correlation with age (Table 1). Further validation against invasively measured pressure, potentially using higher spatial resolution images,[79,80] could determine whether such a tool provides a meaningful estimate of intracranial pressure in a population with relatively minor inter-individual variance.[78]

Figure 7. Measurement of OND and ONSD on MRI. Reproduced from Geeraerts et al.[79]

Box 1 (continued).

Measurement	Interrater agreement (intra-class correlation)	Correlation with age (Pearson's coefficient)
3mm OND	0.77	n/a
3mm ONSD	0.69	n/a
3mm OND:ONSD ratio	**0.58**	**0.31 (rater 1)** **0.30 (rater 2)**
10mm OND	0.58	n/a
10mm ONSD	0.66	n/a
10mm OND:ONSD ratio	**0.36**	**0.12 (rater 1)** **0.27 (rater 2)**

Table 1. Interrater agreement for the OND and ONSD measurements, as well as the correlation of the OND:ONSD ratio with age (*N*=43). OND=optic nerve diameter; ONSD=optic nerve sheet diameter.

Stenotic occlusive disease and heart failure I have already touched upon in the prior paragraph. A potential relation between carotid artery disease and apoplexy was acknowledged already by Hippocrates, and has been followed by a long history of surgical – and later endovascular – amendment of in particular the carotid artery bifurcation.[84] The majority of these interventional studies have aimed at reducing the risk of thromboembolic sequelae, rather than restoring cerebral blood flow, and as such carotid artery desobstruction has been shown effective in reducing risk of recurrent stroke.[85,86] It was already noted at an early stage, and published in a small study in 1976 under the auspicious title "The improvement of cognition and personality after carotid endarterectomy", that cognitive performance may also benefit from carotid surgery.[87] The ipsilateral brain tissue loss in Chapter 2.5 provides further evidence for a role of stenosis in neurodegeneration, along with numerous recent studies – albeit non-randomised and of varying methodological rigour – showing improvement in cognitive performance following carotid artery desobstruction, whether by stenting or endarterectomy, and for symptomatic as well as asymptomatic stenosis.[88-93] In the absence of randomised controlled trials, these should be no basis for routine intervention, but they support aetiological involvement of haemodynamically significant stenosis of the brain supplying arteries, and advocate incorporation of cognitive endpoints in intervention trials of carotid stenosis for stroke prevention in the statin era. Whilst for stroke prevention such interventions depend on the symptomatology of the stenotic disease, the prolonged exposure contributing to neurodegeneration and cognitive decline may well pass unnoticed for a long time, and fit a different paradigm. It thereby remains to be determined whether such associations are the results of (micro)thrombi, and/or cerebral blood flow reduction. The latter may be most visible in the watershed areas on the border of arterial territories, which appear vulnerable to micro-infarction.[94,95] Cortical microinfarcts have recently emerged as a risk factor for cognitive impairment,[96] and although the vast majority currently remains under the

detection limit of in vivo clinical imaging,[97] invisible on 1.5 tesla MRI applied in the Rotterdam Study, it is interesting to speculate about their role in the predominantly frontal – possibly watershed– differences in interhemispheric volumes in Chapter 3.5, and potential mediation of associations of dementia with symptomatic and subclinical heart disease.[98]

Heart failure can be caused by different diseases, such as coronary heart disease, hypertension, and valvular heart disease. While hypertension,[99-104] and coronary heart disease (Chapter 4.1) are risk factors for developing dementia, results in Chapter 4.2 suggest valvular heart disease is not. Nevertheless, if additional reports confirm that dementia risk with coronary heart disease are largely due complications of clinical heart failure,[105] this would call for similar studies investigating mediation of hypertension and more severe valvular heart disease than was subject of investigation in this thesis. Despite the evident disturbance of systemic flow with heart failure, complications are not to arise merely from haemodynamic impairment. Other mechanisms outlined in Chapter 4.1 include thromboembolic complications, shared aetiology including (vascular) amyloid, effects of a pro-inflammatory state (Box 2), or direct effects of natriuretic peptides. Thromboembolism due to secondary arrhythmia,[106] or turbulent blood flow causing brain ischaemia,[107,108] may contribute to cognitive decline and dementia acutely or through repeated subclinical insults. Yet, individual variation in thromboembolic risk is high, and likely attributable to a variety of pro-thrombotic factors. Because of its relevance in incident cardiovascular disease and mortality,[109] I have studied Von Willebrand factor and its main cleavage protein ADAMTS13 in Chapter 4.4. Short-term associations with Von Willebrand factor may indicate a role of endothelial damage rather than a prolonged thrombotic state. Intriguing novel associations of ADAMTS13 with dementia, which mimic ischaemic stroke risk in their interaction with diabetes, provide an incentive for study of independent effects of ADAMTS13 in various manifestations of vascular disease, including dementia. Future studies may look further to identify determinants of high thrombotic risk, such as genetic influence to treatment response,[110,111] and thrombogenic factors like the neutrophil extracellular trap[112] in order to identify patients at high risk, and fit suitable treatment regimens in which benefit outweighs risk for long-term prevention of stroke as well as dementia. Regarding dementia, it is thereby, at least in my view, of the utmost importance to determine whether cerebrovascular pathology stands in any relation at all to the accumulation of amyloid in either the vessel wall or brain parenchyma. In a cross-sectional analysis of the Mayo Clinic Study of Aging, a composite of cardiovascular and metabolic risk factors for cognitive decline was related to neurodegeneration, but not with PET defined amyloid burden. In contrast, mid-life vascular and metabolic risk factors have been associated with ^{18}F-florbetapir uptake in late-life in the population-based ARIC study.[39] My study in Chapter 4.3 suggests that shared effects of amyloid on the brain and systemic vasculature are most likely to arise from

(vascular) amyloid-β40. It is important to note that standardised uptake value ratios of amyloid tracers, whether ^{18}F-florbetapir, ^{18}F-florbetapen, or ^{11}C-PiB, appear to reflect the predominant insoluble form of amyloid,[113,114] which unlike vascular amyloid is mostly amyloid-β42.[115,116] As these are some of the very few studies that have investigated amyloid in relation to vascular disease, more evidence is urgently needed to understand the interplay, or lack thereof, between various pathologies leading to cognitive decline. The suggestion that different amyloid-β isoforms contribute to neurodegenerative pathology differently, and the inability of PET tracers to differentiate between these, emphasises that studies of cerebrospinal fluid markers, covering amyloid and others, in unselected populations remain a challenging, but likely worthwhile undertaking in unravelling the origins of Alzheimer pathology. Other markers could be directed at function and integrity of the cerebral small vasculature, of which I shall provide more detail in the next paragraph.

Box 2. Inflammatory cytokines are an important mediator in the effects of tissue hypoxia. I have outlined various potential mechanisms by which hypoxia can lead to neuronal cell loss in Chapter 3. Reductions in tissue oxygenation can directly trigger expression of various inflammatory cytokines via activation of hypoxia-inducible transcription factors,[117] which may in turn lead to microglia activation and oxidative stress along with release of other pro-inflammatory neurotoxic factors (e.g. TNF-α and IL-1β).[118-120] Of the many cytokines that have been investigated, and found implicated, in Alzheimer's disease in countless preclinical and clinical studies,[120,121] only a handful (and arguably not the most specific) have been assessed in relation to the occurrence of dementia in the population (Table 2). Given the wide implication of the innate immune system in Alzheimer's disease through recent genetic studies,[123-125] assessment of additional cytokines both in population setting seems warranted, and would be particularly interesting against the backdrop of cerebral haemodynamic changes, hypoxia, and cerebral small-vessel disease.[126]

Inflammatory marker	Number of studies	Analysis*	All-cause dementia (HR, 95% CI)	Alzheimer's disease (HR, 95% CI)
C-reactive protein	10	Quantiles	1.37 (1.05-1.78)	1.15 (0.86-1.52)
Interleukin-6	5	Quantiles	1.40 (1.13-1.73)	1.20 (0.94-1.53)
α1-antitrypsine	2	Quantiles	1.54 (1.14-2.08)	1.41 (0.98-2.02)
Lp-PLA2 activity	2	Quantiles	1.40 (1.03-1.90)	1.10 (0.71-1.68)
Lp-PLA2 mass	2	Continuous	1.06 (0.94-1.18)	1.06 (0.93-1.20)
Fibrinogen	2	Continuous	1.27 (1.12-1.44)	n/a

Table 2. Inflammatory markers in relation to incident dementia and Alzheimer's disease. Results from a systematic review and meta-analysis of population-based studies highlighted systemic markers of a pro-inflammatory state that are also often elevated in cardiovascular disease, and provide support for further study of more Alzheimer-specific markers in the community.[127] Lp-PLA2=lipoprotein-associated phospholipase A2; HR=hazard ratio; CI=confidence interval; n/a=not available. *=studies differed in means of exposure classification; results are presented here for the highest versus the lowest quantile.

Throughout this dissertation, cerebral small vessel disease emerges as an underlying cause, effect modifier, or mediator in various of the presented associations. Whether by impairment of neurovascular coupling, hampered nutrient extraction, disturbed blood-brain barrier integrity, aberrant angiogenesis, or amyloid clearance, to name just a few, cerebral small vessel disease exerts important effects on the brain.[129,130] These effects translate into consistent increases in the risk of dementia with cerebral small-vessel disease in the community (Figure 8), while at the same time very little is known about its underlying pathophysiology.[131] The difficulty arises with the agglomeration of pathologies that may be captured under the definition of small-vessel disease, none of which are very well captured on in vivo (Box 3). From endothelial cell and pericyte dysfunction in the tunica intima to impaired vascular smooth muscle cells in the tunica media, and the fragility of the single-cell lumen at the capillary level; these are all amassed in a handful of all-encompassing MRI markers. And even what we appreciate there is just a tip of the iceberg of disarray in the cerebral white matter.[132] Diffusion imaging now takes us to the next level of detection, with abnormalities in microstructure emerging as soon as middle-age in relation to cardiovascular

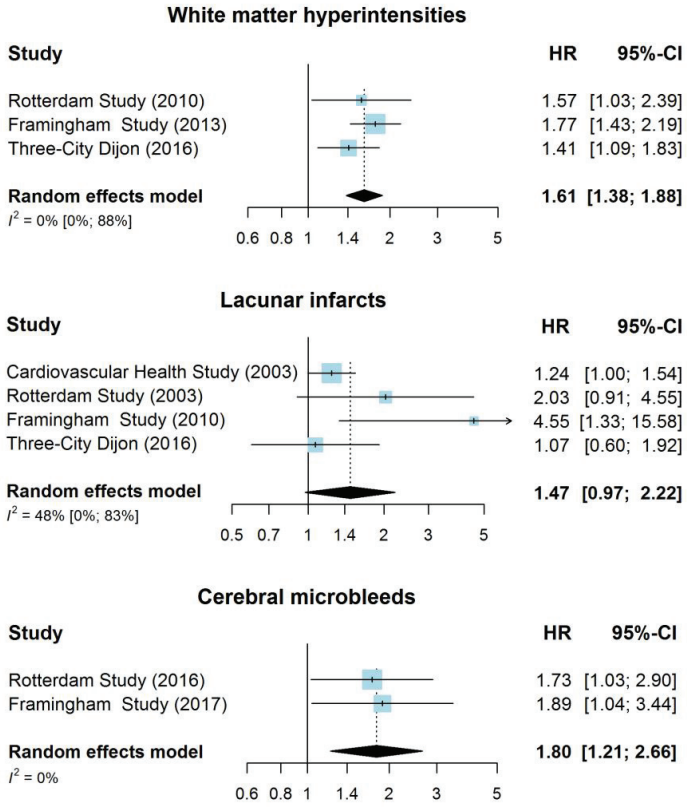

Figure 8. Association between cerebral small-vessel disease and risk of dementia, on the basis of all published population-based studies.[128] HR=hazard ratio; CI=confidence interval.

risk factors (Figure 9), closely related to changes in amyloid-β42 in the cerebrospinal fluid.[133,134] It begs the question how these microstructural abnormalities relate to preclinical observations of neurovascular unit dysfunction, blood-brain barrier disintegration, and demyelination. The importance of the blood-brain barrier covers many processes, as has been extensively discussed elsewhere,[129] but I shall highlight certain mechanisms relating to cerebral blood flow and haemodynamic response. From pathology it is known that cerebral small-vessel disease consist of atherosclerosis, hyaline deposition (lipohyalinosis), and fibrotic changes with arteriolosclerosis.[135] Overexpression of hypoxia induced factors suggests involvement of chronic hypoperfusion,[136] but evidence from a small number of longitudinal studies is conflicting about whether hypoperfusion precedes or is a consequence of white matter changes.[137] The former is plausible, as vital components of the neurovascular unit, notably pericytes and vascular smooth muscle cells, are implicated in white matter disturbance and neurodegeneration,[138,141] as well as progression of cerebral amyloid angiopathy.[142] This lends support to the idea that neurovascular dysfunction can lead to accumulation of amyloid-β, which in turn enhances vascular and neuronal damage due to its toxic effects.[143] Whether in the initial stages, or as a consequence of disease, these processes may be influenced by endothelial activation, inflammation, (aberrant) angiogenesis, and capillary dysfunction,[121,144,145] which could all leave there mark on the conglomerate of *small-vessel disease* seen on in vivo MRI. Recent advances in neuroimaging may allow a more detailed impression of metabolism, blood flow, and blood-brain barrier permeability to facilitate insight in the pathophysiology of cerebral small-vessel disease.[146] At the same time, a closer look at long established methods may also shed light on previously underappreciated differences in for example patterns of white matter hyperintensities on MRI,[147] and the paradox between dementia risk and small-vessel disease among *APOE* ε2 carriers.[148] If we succeed in enhancing a two-way interaction between preclinical and clinical study design, linking abovementioned observations from lab to population, I am certain that such studies will aid greatly in our understanding of the cerebrovascular contribution to dementia.

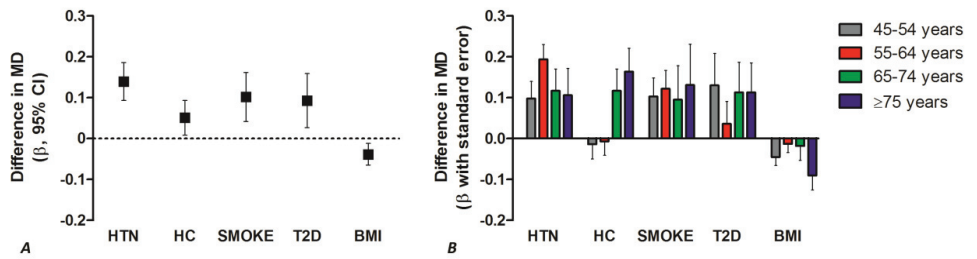

Figure 9. Risk factors for impaired white matter integrity, as measured by mean diffusivity. Detrimental effects of risk factors on white matter integrity are already measurable in mid-life. HTN=hypertension; HC=hypercholesterolaemia; SMOKE=current smoking; T2D=type 2 diabetes; BMI=body mass index per 5 points increase. (Cremers LGM & Wolters FJ, 2017)

Box 3. Current techniques for in-vivo imaging of blood vessels fall short in visualisation of the cerebral small vasculature. Computed tomography (CT) and magnetic resonance imaging (MRI) allow imaging of large arteries and arterioles down to 100-300µm in diameter, but to really understand the pathophysiological processes underlying small-vessel disease more fine-grained insight in the microcirculation, with its capillaries of generally <20 µm in diameter, is needed. Albeit not applied directly to the brain, such insight might come from in-vivo imaging of the microvasculature using sidestream dark field imaging (SDF). SDF is as a rapid, non-invasive imaging method, that allows direct visualisation of submucosal capillary beds by emitting light at a frequency optimal for absorption by deoxy- and oxyhemoglobin in erythrocytes (Figure 10).[149] SDF-derived measures have recently emerged as a marker of microvascular health in patients with diabetes,[150] undergoing cardiac surgery,[151] or at the intensive care unit.[152] Using the MicroScan SDF imaging device (MicroVision Medical, Amsterdam, the Netherlands) for sublingual measurements I have, in highly appreciated collaboration with Drs. S. Sedaghat and S. Licher, assessed intra-rater reliability on 20 healthy young volunteers, and feasibility of the method in pilot study in the Rotterdam Study cohort. Intra-rater reliability was reasonable for small vessels, but poor for the larger vessels within the capillary bed (i.e. small arterioles to large capillaries) (Table 3), which may reflect high within subject variability, but could also indicate low between subject variability potentially causing it to take on negative values. In a healthy population, this is not unlikely, and this may be one of the limitations of the methodology, compared to its previous applications in patients with more severely impaired microcirculation. In the feasibility study, SDF imaging was applied to 50 consecutive Rotterdam Study participants at the research centre after an initial training period of several weeks. Upon systematic grading of the image quality,[153] however, there were problems with stability, and to a lesser extent focus and applied pressure (Figure 11).

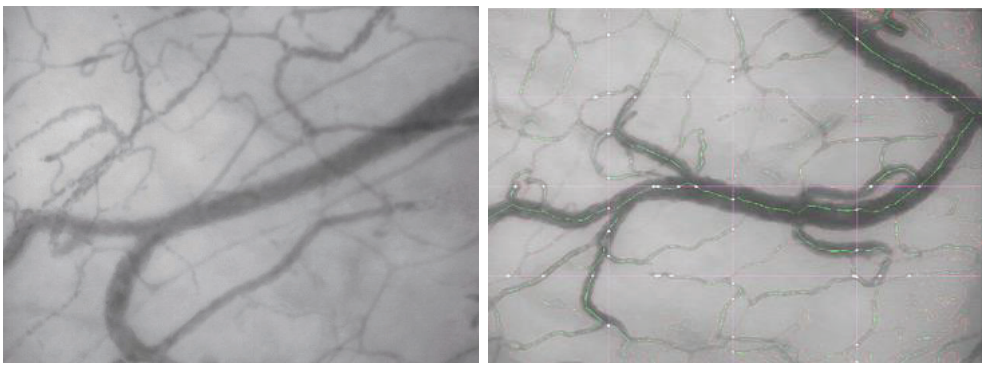

Figure 10. SDF-image of the submucosal capillaries. The left panel shows a sublingual measurement in a healthy individual. In the right panel, automated off-line detection of the vessels in the captured image is seen (AVA Software version 4, MicroVision Medical BV, Amsterdam, the Netherlands).

Box 3 (continued).

	Intra-rater #1	Intra-rater #2	Inter-rater
Number of crossings	-0.30 (-2.4,0.51)	0.13 (-1.42, 0.69)	0.09 (-1.3, 0.64)
DeBacker density	-0.30 (-2.4, 0.51)	0.13 (-1.42, -0.69)	0.09 (-1.3, 0.64)
Perfused number of crossings	-0.10 (-1.89, 0.58)	0.002 (-1.78, 0.65)	0.17 (-1.09, 0.67)
Perfused DeBacker density	-0.10 (-1.89, 0.58)	0.002 (-1.78, 0.65)	0.17 (-1.09, 0.67)
Proportion perfused vessels	0.28 (-0.89, 0.73)	-1.17 (-15, 0.77)	-0.02 (-1.92, 0.64)
Number of crossings (small)	0.79 (0.44, 0.92)	0.55 (-0.24, 0.84)	-0.17 (-1.97, 0.54)
DeBacker density (small)	0.79 (0.44, 0.92)	0.55 (-0.24, 0.84)	-0.17 (-1.97, 0.54)
Perfused number of crossings (small)	0.77 (0.38 – 0.91)	0.57 (-0.21, 0.85)	-0.05 (-1.66, 0.58)
Perfused DeBacker density (small)	0.77 (0.38 – 0.91)	0.57 (-0.21, 0.85)	-0.05 (-1.66, 0.58)
Proportion perfused vessels (small)	0.56 (-0.15, 0.84)	-0.62 (-5.7, 0.63)	0.18 (-1.26, 0.70)

Table 3. Intra-rater and interrater agreement for several SDF imaging parameters. Automated quantification was done using AVA Software version 4 (MicroVision Medical, Amsterdam, the Netherlands). Values are the intra-class correlations, for the interrater agreement presented for the means of two readings.

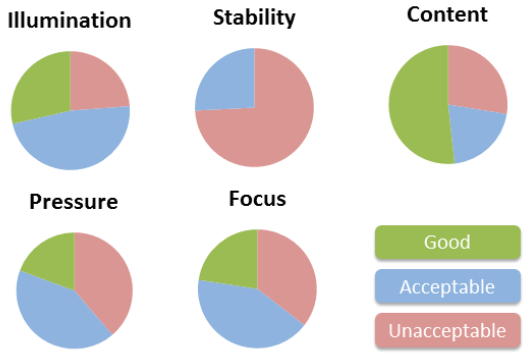

Figure 11. Assessment of image quality from the captured MicroScan images of a random subset of 30 Rotterdam Study participants. Images were graded according to a previously published quality score.[153] Illumination relates to brightness and contrast; Focus to sharpness in the region of interest; Content to determination of the types of vessels imaged; Stability to frame motion that can be adequately stabilised without blur; Pressure to iatrogenic mechanical pressure causing misrepresentation of flow.

Continuous work may solve part of the stability issue by using a stable treatment chair, and a foot pedal rather than a mouse button to capture the image on scope. Additionally, several additional captured frames for image selection may improve image quality, and improvements in automated segmentation with new software releases may further improve consistency. All in all, this pilot approach reveals that a number of challenges pertaining SDF imaging need solution in further studies in order to derive such reliable and consistent parameters of the microcirculation that the method is feasible for use in unselected population of community-dwelling individuals, in whom within-subject variability needs to be minimised to detect meaningful between-subject variability.

A genetic basis for risk prediction

In the above discussion of aetiology, I have several times staged a genetic predisposition to dementia, notably *APOE*, as an important tool in identifying preclinical changes and unravelling disease aetiology. Its second useful purpose, which is mainly contemplated in Chapter 5, I shall address here. The long preclinical disease phase of dementia, in combination with the failure of numerous trials that enrolled patients in the symptomatic later stages of disease,[154,155] has led to an urge for earlier intervention, with several prevention trials underway.[30,156,157] The feasibility of such trials largely depends on the ability to recruit individuals early, but nevertheless at such a stage that clinical decline can be observed during the trial period. One strategy to achieve this is to focus on individuals at high genetic risk, and several trials now use genetic data for inclusion of individuals at high risk of dementia (e.g. DIAN (ClinicalTrials.gov Identifier NCT01760005) and the Generation Study (NCT02565511)). Despite this expeditious attitude, very few studies have in fact documented prospective, absolute risks of developing dementia, or mild cognitive impairment for that matter. The *APOE*-associated risks I describe in Chapter 5.1 are substantially lower than previously reported estimates on the basis of cross-sectional and case-control data, and markedly higher in a convenience cohort than in representative samples of the general population. The pool of eligible trial participants for any particular trial will determine which estimates are most suitable for the situation at hand, but in any case, accounting for characteristics of the source population is vital to trial design and reliably informing potential participants. Additional studies providing prospectively derived absolute risks with varying sampling strategies, clinical assessment methods, and population characteristics are critical to developing the best possible answers for clinical trial design. As reference data from the general population are – at least in Europe and North America – often already in store, this is an area in which pharmaceutical industry may well work in concert with academia for advancement of trial recruitment and timely results.

Any trial using genetic eligibility criteria while aiming to retain meaningful generalisability of its findings should include *APOE* in its sampling strategy, but with the increasing number of identified common risk variants, it need not be *limited* to *APOE*. Since the first genome-wide significant loci for Alzheimer's disease were identified in 2010,[158] over 20 common genetic variants followed. As shown in Chapter 5.4 current insight in genetic risk can already make valuable contributions to risk stratification for dementia in the general population, as was previously suggested on the basis of discrimination between cases and controls,[159] and relative hazards from a joint analysis of several prospective cohort studies.[160] Yet, much of the heritability of dementia remains unaccounted for. Yielding whole genome data from 29 Alzheimer disease centres in the United States, it was estimated that about 50% of phenotypic variance is explained by genetics, of which half is accounted for by *APOE*, and

another 5% by the other current genetic discoveries.[161] Although the predictive value of genetics may be enhanced by the use of polygenic risk scores,[162,163] it thus seems that, at least for the moment, a simple family history is a valuable tool for risk stratification in research and most certainly clinical setting. Information about age at onset in relatives is thereby indispensable, and I believe that the evidence from Chapter 5.3, along with a prior modelling study,[164] provides the evidence needed for its incorporation in routine clinical practice, with an age cut-off at (the pragmatic limit of) about 80 years.

Whilst genetic risk prediction will be refined by identification of further risk variants, substantial additional improvement of prediction could be achieved by accounting for sources of inter-individual variability in gene expression. The predictive value of genetic variants may vary widely on an individual basis due to changes in transcription or translation. Although extensive discussion of gene-environment interactions, epigenetics, and microRNAs is beyond the scope of this dissertation, increasing insight in these phenomena could benefit both understanding of aetiology and risk prediction. Additionally, more precise prediction of disease may be achieved by measuring gene products in the circulation. This is exemplified in Chapter 5.5 with associations of plasma apolipoprotein E (apoE) independent of the *APOE* genotype, and notably of discriminative value in heterozygous *APOE* carriers who have noticeably wider varying expression.[165] Other studies underline the quantitative importance of apoE in lipid metabolism and onset of dementia and ischaemic heart disease.[166] Interestingly, apoE is one of relatively few lipoproteins present both in plasma and cerebrospinal fluid. As the central nervous system does not use triglycerides as an energy source, and receptor-mediated transcytosis of apolipoproteins occurs across the blood-brain barrier into the systemic circulation,[129] apoB containing (very) low- and intermediate-density lipoproteins are absent in the healthy brain. This leaves it reliant on 10 lipoproteins in high-density lipoprotein (HDL)-like particles, compared to 85 different proteins in plasma HDL.[167] Aside apoE, this includes apoA-I, apoA-II, apoA-IV, apoD, apoC-II, apoC-III, apoC-IV, apoH, and apoJ (clusterin). Their change with age, or role in health and disease remains much unknown. Although apoE is the predominant apolipoprotein in the brain, it is interesting to speculate whether measurement of various specific lipoproteins might explain inconsistencies in the literature regarding the association of traditionally measured lipid fractions with dementia,[168] and may provide a conglomerate of accessible biomarkers to aid in risk prediction of dementia. The recently reported associations of several peripheral metabolites, notably HDL fractions, with cognitive performance and dementia risk in that respect opens an interesting avenue.[169]

With the seemingly fast approaching implementation of genetics for clinical risk stratification, it is important to also acknowledge its impending mainstream application in direct-to-consumer genetic testing. Manufacturers like 23andMe currently only includes carriership of the *APOE* ε4 allele in their genetic risk result for late-onset Alzheimer's disease, but results like in Chapter 5.4 may change that stance on the basis of consistent reports in only a few population-based studies. Against the backdrop of this wide access to genetic information, it is important to advocate the preventability of dementia, and discourage a general conception of the disease as the pending sword of Damocles (Figure 5). Theoretically, even the pathogenesis of a completely heritable disease could occur solely through gene-environment interactions, and some (more subtle) examples can be appreciated for instance in shared genetics between body mass index and cognition.[170] As for trial eligibility, it is my opinion that any gain in power by selection of trial participants on the basis of (genetic) risk should be carefully weighed against the potential reduction of generalisability. Whilst feasibility warrants a certain degree of pragmatism, this should not withhold continuous observation of trial participants and other open-label use, and serious consideration of the benefits to a wider population before the chicken with the golden eggs goes to market.[171] And with that, it is high time that I move on towards a critical assessment of the foundation upon which these aforementioned statements are made.

METHODOLOGICAL CONSIDERATIONS

"Most published research findings are false".[172] The infamous quote by John Ioannidis was accompanied by simulations showing that in most study designs and settings, a research claim is more likely to be false than true. Sadly his estimations turned out not far from the truth, with about half of psychological research reports failing to replicate;[173] a statistic that is likely even worse for cognitive neuroscience.[174] Many of the same perils threaten dementia research: an myriad of small studies testing a great number of potential pathways and relationships, using rather few standards in the use of biomarkers, in the face of immense financial interests and commitment to longstanding theories in an overheated research area. How many of the findings presented in this dissertation will stand the test of time? Fortunately, not all is lost. The most important determinants of a study's positive predictive value are the a priori probability (i.e. a well-founded hypothesis), and the (elimination of) bias.[172] To the ears of an epidemiologist, such notions sound like perfect symphony. Keeping in mind that methods are never more than a means to an aim, some consideration of their use, misuse, and future use is certainly in place.

Study population and design

Salus populi suprema lex esto. – Cicero, *De Legibus Book III*

All the original research in this dissertation is embedded in one or multiple population-based cohort studies. The importance of the study setting is I think best illustrated by the average age at diagnosis of dementia in the population. Of Rotterdam Study participants who developed dementia, the median age at diagnosis was 84.0 years (Figure 12), which is substantially higher than the age of most participants in clinical studies. Elderly individuals, particularly those in nursing homes, get omitted from many studies due to referral bias, and tertiary centres which are most prolific academically, tend to specialise in young onset dementia with patients presenting not seldom before age 65. Is it truly the *welfare of the people* that is served? Given the accumulation of various pathologies in the elderly brain, a 60-year old patient with dementia is likely incomparable to their 85-year old counterpart in most ways if it comes to aetiological (or even diagnostic and prognostic) study. Although this renders findings from population studies more generalisable to the wider source population, it should be noted that the lack of ethnic and socioeconomic diversity in the Ommoord area might still limit applicability of results outside the Rotterdam Study population.

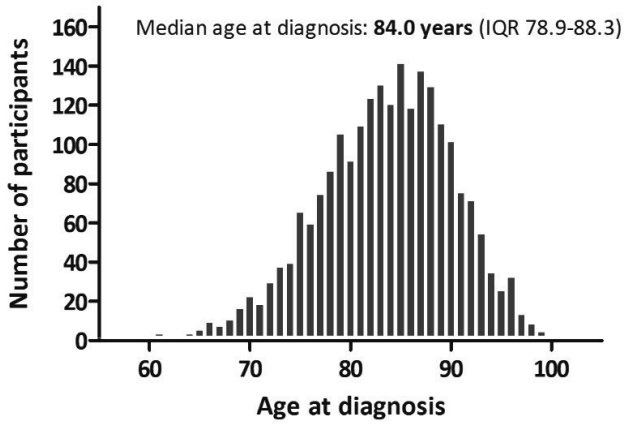

Figure 12. Age at onset of dementia in the population, based on data from the Rotterdam Study between 1990 and 2016.

Next to generalisability, the population-based design of presented studies limits potential selection bias. The average response rate of 72% in the Rotterdam Study is, in that respect, still not perfect, but comparable to other population studies like the Framingham Heart Study, and much higher than in contemporary biobanks such as the UK Biobank with a response rate of less than 10%.[175] Moreover, the wide range of invitees renders participation less related to the exposure and outcome of interest. Evermore important than selection at baseline is the completeness of follow-up. I consider the extensive follow-up data collection

of the Rotterdam Study one of its main advantages for dementia research, and ergo one of the strongpoints of this thesis, in particular because the various means of disease ascertainment – as eluded to below – generally limit attrition to less than five percent. This, however, does not apply to the measures that solely rely on repeated centre visitation. Despite the heart-warming dedication of the Rotterdam Study participants, some attrition during the course of the four-yearly examination cycle is inevitable. We observe about 20% attrition per cycle, which is generally related to baseline exposure (e.g. Chapter 3.1). Subsequent selection bias may thus have altered – and most likely attenuated – effect estimates. The only way of preventing this bias would be by more frequent in-person examinations, but incremental costs, research centre capacity, and burden on participants cause higher frequency examination cycles unfeasible. That is why, with the exception of the phenomenal yearly participation rates in the (somewhat smaller) Rush Memory and Ageing Project and Religious Orders Study,[176] participants of large population-based cohorts revisit at most once every four years. Nevertheless, we have now arrived at a stage that three and at times even four consecutive measurements of notably cognition are available in the Rotterdam Study (e.g. Chapter 3.3 and 4.4), with analytical techniques like linear mixed models accounting for attrition to some degree. Yet, it is not just attrition that hampers use of these tests.

Measuring cognition

Following the failures of several dementia prevention trials in the late 1990s and early 2000s, clinical trials started to determine the effect of interventions on cognition as a more sensitive, continuous outcome measure than dementia.[29] Changes in cognitive test performance can more readily detect a role of determinants in the long pre-symptomatic neurodegenerative disease course (thus rendering reverse causation less likely). From a population perspective, it furthermore captures the burden of cognitive impairment beyond that of dementia only. Cognitive test results were therefore assessed and reported in every chapter for which they were measured along with the exposure of interest, and thus make up an important pillar of the presented findings. Yet, cognitive performance is versatile. Subject to large within person variability, whether due to time of day,[177] sugar or caffeine consumption,[178] or a good night's rest,[179] true effects may easily be obscured. Any gain in accuracy of cognitive measures should therefore be applauded.

With the aim of improving measurement accuracy, I have made minor adjustments to the analysis plan along the course of this research undertaking. To maximise the yield from the test of manual dexterity (i.e. the Purdue pegboard), I have turned to using the sum score of three attempts (dominant hand, non-dominant hand, and both) rather than a single score only. Furthermore, a prudent time-penalty to account for error in the Stroop task resulted in

another inconsistency between chapters. I thereby adopted the "arbitrary but […] justified by the situation" plan of correction proposed by John Ridley Stroop in his original 1935 paper.[180] Although differences between the adjusted and unadjusted scores are in practice modest, I believe the rationale favours the more elegant error adjustment, and this has now been adopted as the standard approach within the Rotterdam Study. As a third matter of inconsistency, the included tests somewhat differed between chapters, as memory testing and manual dexterity were only added to the core protocol from the fourth examination cycle onwards, whereas executive function and information processing were already incorporated one cycle earlier. Although the exact reasons never became fully clear to me, I suspect it relates to the belief at the time that executive dysfunction was rather specific for vascular cognitive impairment, whereas memory would predominantly be affected in Alzheimer's disease.[181] Findings across cognitive domains in this thesis, along with many other studies, in my view support a more nuanced outlook. Certainly, pathology that prominently features in specific brain regions may give rise to specific symptoms, and certain cognitive screening tests may be more suitable than others for say vascular cognitive impairment,[182] but the multitude of pathologies often underlying late-onset dementia cases merit assessment of a range of cognitive domains.

This leaves us with a conglomeration of cognitive tests, which despite their differences show substantial correlation amongst themselves (Table 4). That notion led English psychologist Charles Spearman (1863-1945) to believe that disparate cognitive test scores largely reflect a single ´general intelligence factor', or *g*-factor.[183] In his seminal 1904 paper, he wrote that "all branches of intellectual activity have in common one fundamental function, whereas the remaining or specific elements of the activity seem in every case to be wholly different from that in all the others". Spearman spearheaded the use of factor analysis, and it may be seen as a tribute to his work that the *g*-factor features in this thesis. The factor analysis generally explained about 50% of variance in cognitive test scores in the Rotterdam study sample, in line with observations of child and adolescent intelligence.[184] While providing a more robust measure of cognitive performance, the *g*-factor, as I think rightly emphasised by contemporaries of Spearman, also entails a devaluation of specific abilities. More recent insight suggests indeed that different components of intelligence have their substrate in distinct neural networks, with the higher-order *g*-factor recruiting multiple of these.[185] In light of such specific networks, incorporation of a motor function tests, be it gait or dexterity, can add important information with regard to neurodegenerative pathology, as we have substantiated by showing independent associations of motor function with dementia and parkinsonism (Figure 13).[186]

	Letter-digit	Verbal fluency	Stroop	Word learning	Purdue
Letter-digit		.46	.52	.39	.45
Verbal fluency	.46		.38	.40	.30
Stroop	.52	.38		.33	.36
Word learning	.39	.40	.33		.27
Purdue	.45	.30	.36	.27	

Table 4. Correlation between cognitive test scores during the fourth examination cycle of the Rotterdam Study, expressed as Pearson's correlation coefficient. For the 15-word learning task, delayed recall is depicted, and for the Purdue pegboard the sum score of all three attempts.

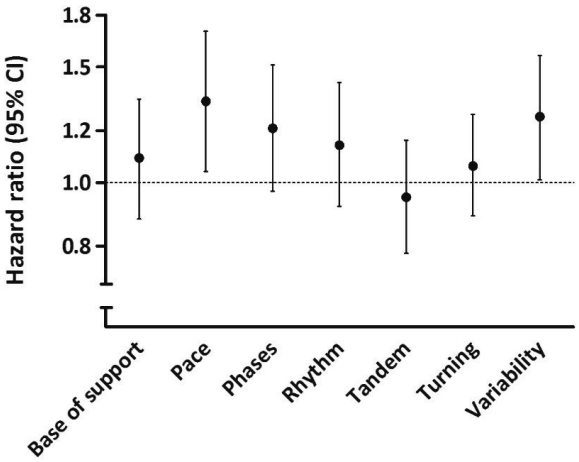

Figure 13. Gait domains and incident dementia. All gait parameters were standardised, and higher scores correspond to worse gait (Darweesh SKL, Wolters FJ, Licher S, et al. Submitted for publication).

Other limitations to the cognitive assessment battery persist, including aforementioned attrition and sources of within subject variability. The latter warrants further study to determine the magnitude and potential gain by harmonizing for instance time-of-day in repeated assessment. Although the four-year interval in the Rotterdam Study limits learning effects, the population-based inclusion of healthy and younger individuals renders ceiling effects all the more relevant. For this reason – and the fact that it was intended as a screening tool – I have refrained from using the mini-mental state examination as an outcome measure in this thesis. A more extensive skillset could be useful, particularly in younger study participants, provided its feasibility within the population-based setting.

In view of these deliberations and impediments, it almost comes as a surprise that I generally observed consistent associations with cognitive decline in non-demented individuals, as compared to incident dementia. This supports the notion of a continuum of neurodegenerative brain pathology in the population rather than dementia as a bimodal disease entity.[187] It should be noted that effect estimates of exposures on cognitive test performance were generally small, yet all but negligible in light of observed effects of the

APOE ε4 allele of about 0.10-0.15 standard deviations (per allele) per 10 years. In my view, and I think many would agree, the effect of *APOE* is more substantial than reflected by this number. It underlines that much may be gained by eliminating sources of within subject variability, along with minimising attrition, more sensitive cognitive tests, and improved statistical methods for longitudinal data analysis. Application of technology, for instance by tools for repeated cognitive assessments on a tablet computer, designed to minimise learning effects, could in that respect be an important step forward.

Defining and identifying dementia

Although systematic classification of diseases had been attempted in one form or another for some centuries, the first formal, universally accepted classification saw the light in 1893, when Jacques Bertillon introduced the *Bertillon Classification of Causes of Death*.[188] Post aut propter, the habit of using eponyms died out soon after. Bertillon's classification was based on an earlier model by British epidemiologist William Farr, classifying disease chiefly by their anatomic site. Advocated by Farr, the classification was gradually expanded to also include morbidity, eventually leading to the *International Statistical Classification of Diseases and Related Health Problems*, in short the *International Classification of Diseases (ICD)*, as endorsed by the first World Health Assembly in 1948.[188] The World Health Organisation thereby took over the responsibility for subsequent revisions from the International Statistical Institute and the Health Organization of the League of Nations. The 1948 edition (officially the sixth revision) was the first to include a section on mental disorders, largely inspired by Emil Kraepelin´s nosology of psychiatric disease,[189] which had dementia as a neurodegenerative disorder acknowledged among the *Psychoses* (Table 5). Meanwhile, inspired by the lack of disease description in the ICD, the American Psychiatric Association developed a variant on the ICD-6 that was first published in 1952 as the first edition of the *Diagnostic and Statistical Manual of Mental Disorders (DSM)*. It was the first to focus on clinical rather than administrative use.

The purpose of this brief historical disquisition is to highlight the continuous changes in diagnostic subtypes, while since the early 1980s the symptoms required for classification as dementia (be it *major neurocognitive disorder* in DSM-V) remained virtually unaltered. This is highly relevant for longitudinal studies, in particular when trying to establish time trends in the incidence of disease, as I have endeavoured in Chapter 2.3. A second observation of the table shows the discrepancy in detail between the DSM and ICD classifications. These different diagnostic criteria can give rise to large differences in dementia diagnosis, with prevalence reported 10-fold higher when using DSM-III criteria versus ICD-10 coding.[190] Such differences can have a large impact on registry studies, which often rely exclusively on ICD coding for obtaining clinical diagnoses. We saw an example of this in Chapter 4.1, in which

the share of dementia diagnosis comprising Alzheimer's disease was three- to four-fold lower in registry studies compared to population-based cohort studies. Third, the changes in classification show that, similar to the aforementioned changes in paradigm of cognition, memory has moved from the predominant feature of dementia to one of multiple potential cognitive deficits. Although clinical presentation may often remain driven by memory impairment, equal attention for other domains likely improves detection of the burden of cognitive impairment across age groups in the population.

The syndrome-based diagnosis of dementia (i.e. all-cause dementia), rather than aetiological subtypes, has been the primary outcome measure in all of the studies in this dissertation. Distinguishing Alzheimer's disease clinically from other dementia subtypes such as vascular dementia, dementia with Lewy bodies, or dementia with Parkinson's disease, has proven challenging, if not impossible in the light of the multitude of pathologies that co-occur in the elderly population. This is particularly troubling since over 90% of dementia patients at the population level are diagnosed after the age of 70 years (Figure 12). Consequently, population-based studies of dementia generally face patients in whom a large number of factors contribute to cognitive decline and dementia onset. This highly multifactorial aetiology has long hampered robust definition of dementia subtypes based on clinical phenotype, and consensus about what defines Alzheimer's disease in the population is lacking still. Yielding NINCDS criteria for clinical Alzheimer's disease, the share of dementia cases classified as Alzheimer's disease was very similar among population-based studies in this thesis, but not in proportion with for example registry data, and not necessarily reflective of underlying presence of hallmark Alzheimer pathology. Currently, definitions of disease subtypes are based in part on the presence or absence of risk factors, with a strong emphasis on cerebrovascular disease. Defining a subtype based on a determinant (aetiology-based diagnosis) precludes proper investigation of these – or related – determinants. For instance, if a diagnosis of Alzheimer's disease is conditioned on the absence of cerebrovascular disease, it is likely that effects on Alzheimer's disease of risk factors that are associated with cerebrovascular pathology are spuriously not detected. By contrast, a syndrome-based diagnosis of dementia can be defined with high consistency across studies. In studies that incorporate imaging- or cerebrospinal fluid-based markers of underlying pathologies (e.g. amyloidopathy, vascular lesions), it is possible to quantify how much of the effects of risk factors on all-cause dementia are mediated by each pathology. However, such studies are turning feasible only in recent years, and longitudinal imaging data to address these issues are eagerly awaited.

With a uniform definition of the syndrome, the challenge remains to diagnose individuals in society. Means of dementia ascertainment vary widely between studies, from routinely

Diagnostic and Statistical Manual of Mental Disorders (DSM)		International Classification of Diseases (ICD)	
DSM-V (2013)	**Major neurocognitive disorder** A. Evidence of significant cognitive decline from a previous level of performance in one or more cognitive domains: Learning and memory; Language; Executive function; Complex attention; Perceptual-motor; Social cognition. B. The cognitive deficits interfere with independence in everyday activities. At a minimum, assistance should be required with complex instrumental activities of daily living, such as paying bills or managing medications. C. The cognitive deficits do not occur exclusively in the context of a delirium D. The cognitive deficits are not better explained by another mental disorder (e.g. major depressive disorder, schizophrenia) Any aetiological subtypes of major neurocognitive disorder are not specifically mentioned.	**ICD-10 (2016)**	**Dementia** (F00-F03) is a syndrome due to disease of the brain, usually of a chronic or progressive nature, in which there is disturbance of multiple higher cortical functions, including memory, thinking, orientation, comprehension, calculation, learning capacity, language, and judgement. Consciousness is not clouded. The impairments of cognitive function are commonly accompanied, and occasionally preceded, by deterioration in emotional control, social behaviour, or motivation **Alzheimer disease** (F00) is a primary degenerative cerebral disease of unknown aetiology with characteristic neuropathological and neurochemical features. The disorder is usually insidious in onset and develops slowly but steadily over a period of several years. **Vascular dementia** (F01) is the result of brain infarction due to vascular disease, including hypertensive cerebrovascular disease, with usually small infarcts but cumulative in effect.
DSM-IV-TR (2000) **DSM-IV (1994)**	**Dementia** A. Development of multiple cognitive deficits manifested by both: 1. Memory impairment 2. At least one of the following: Aphasia; Apraxia; Agnosia; Disturbance in executive functioning B. The cognitive deficits in A1 and A2 each cause significant impairment in social or occupational functioning and represent a significant decline from a previous level of functioning C. The cognitive deficits do not occur exclusively during the course of delirium **Alzheimer´s disease** is further characterised by gradual onset and continuing decline, not due to any other systemic (e.g. vitamin deficiency) or central nervous system disorder (e.g. Parkinson´s disease, cerebrovascular disease). A diagnosis of vascular dementia requires the presence of focal neurological signs, or evidence of cerebrovascular disease on imaging judged to be etiologically related to the disturbance.	**ICD-10 (2003)**	**Dementia** (F00-F03) is a syndrome due to disease of the brain, usually of a chronic or progressive nature, in which there is disturbance of multiple higher cortical functions, including memory, thinking, orientation, comprehension, calculation, learning capacity, language, and judgement. Consciousness is not clouded. The impairments of cognitive function are commonly accompanied, and occasionally preceded, by deterioration in emotional control, social behaviour, or motivation. This syndrome occurs in Alzheimer's disease, in cerebrovascular disease, and in other conditions primarily or secondarily affecting the brain. **Alzheimer's disease** (F00) is a primary degenerative cerebral disease of unknown aetiology with characteristic neuropathological and neurochemical features. The disorder is usually insidious in onset and develops slowly but steadily over a period of several years. **Vascular dementia** (F01) is the result of brain infarction due to vascular disease, including hypertensive cerebrovascular disease, with usually small infarcts but cumulative in effect.
DSM-III-R (1987) **DSM-III (1980)**	**Dementia** A. A deterioration of previously acquired intellectual abilities of sufficient severity to interfere with social or occupational functioning. B. Memory impairment C. At least two of the following: Impairment of abstract thinking; Other cognitive deficits such as impaired calculations, apraxia, or anomic aphasia; Impairment in judgment; Impairment in impulse control; Personality change. D. Does not meet criteria for Intoxication or Delirium, although these may be superimposed. E. Either of the following: (1) Evidence from physical exam, laboratory tests, or history of a specific organic factor that is judged to be etiologically related to the disturbance or (2) in the absence of such evidence, an organic factor necessary for the	**ICD-9 (1977)**	**Senile and pre-senile organic psychotic conditions** (290) - **Senile** (290.0) - **Pre-senile** (290.1) - **Senile depressed/paranoid** (290.2) - **Senile with acute confusional state** (290.3) - **Arteriosclerotic dementia** (290.4) - **Other** (290.8) or **unspecified** (290.9). No further disease descriptions are provided, nor is there any mention of Alzheimer's disease as a specific disease entity.

	development of the syndrome can be presumed if the behavioral change represents cognitive impairment in a variety of areas and if conditions other than the Organic Mental Disorders have been reasonably excluded. Subtypes included primary degenerative dementia, multi-infarct dementia, alcoholic dementia, dementia post head trauma, dementia post anoxia, dementia associated with specific neurological disease, undiagnosed dementia.		
DSM-II (1968)	**Senile and pre-senile dementia (290)** - **Senile dementia** (290.0): This syndrome occurs with senile brain disease, the causes of which are largely unknown. The category does not include the pre-senile psychoses nor other degenerative diseases of the central nervous system. While senile brain disease derives its name from the age group in which it is most commonly seen, its diagnosis should be based on the brain disorder present and not on the patient's age at times of onset. Even mild cases will manifest some evidence of organic brain syndrome: self-centeredness, difficulty in assimilating new experiences, and childish emotionality. Deterioration may be minimal or progress to vegetative existence. - **Pre-senile dementia** (290.1): This category includes a group of cortical brain diseases presenting clinical picture similar to those of senile dementia but appearing characteristically in younger age groups. **Alzheimer's** and **Pick's diseases** are the two gest known forms, each of which has a specific brain pathology. When the impairment is not of psychotic proportion the patient should be classified under *Non-psychotic Organic Brain Syndrome with senile or pre-senile brain disease*.	**ICD-8 (1965)**	**Senile and pre-senile dementia** (290) - **Senile dementia** (290.0) - **Pre-senile dementia** (290.1)
DSM-I (1952)	**Chronic Brain Syndrome associated with senile brain disease.** **Alzheimer's disease** was classified as *Chronic Brain Syndrome with other disturbance of metabolism*, whereas **Pick's disease** was *Chronic Brain Syndrome associated with disease of unknown cause*.	**ICD-7 (1955)**	No mention of dementia, but under **Psychoses** (300-309): - **Senile psychosis** (304) - **Pre-senile psychosis** (305) - **Psychosis with cerebral arteriosclerosis** (306)
		ICD-6 (1948)	No mention of dementia, but under **Psychoses** (300-309): - **Senile psychosis** (304) - **Pre-senile psychosis** (305) - **Psychosis with cerebral arteriosclerosis** (306)

Table 5. A historical overview of the DSM and ICD classification of dementia. The specific edition of the nosological scheme is provided along with its year of publication, and the code of individual diagnoses if applicable. Minor changes in the text revision (DSM-IV-TR) and revision (DSM-III-R) of the DSM-IV and DSM-III are not included in the table for simplicity. Subtypes are provided for illustration rather than completion.

collected healthcare data to frequent meticulous cognitive assessments per study protocol. For the Rotterdam Study, information from in-person screening was supplemented by data from the electronic linkage of the study database with medical records from all general practitioners and the regional institute for outpatient mental health care. In the Dutch healthcare system, the entire population is entitled to primary care that is covered by their (obligatory) health insurance. The general practitioner functions as a 'gate-keeper' for referral to secondary and tertiary care providers, who are required by law to report back to the referring general practitioner about test results and clinical diagnoses. With this linkage, the entire cohort is thus continuously monitored for detection of interval cases of dementia between centre visits. The combination of these two modalities improves sensitivity *and* specificity, compared to reliance on for example death certificates or registry data. Sensitivity and specificity of dementia diagnosis on the basis of ICD coding range between 8-87% and 57-100%, respectively,[191] whereas sensitivity of death certificates for a diagnosis is no higher than 54%.[192] To ensure accurate interpretation, this needs to be taken into consideration in study design and interpretation of results obtained using routinely collected data. Conversely, in studies that rely solely on re-examination for diagnosis, sensitivity may rapidly decrease with more prolonged intervals and high loss to follow-up. Although analytical methods like illness-death models may in part account for the interval censoring, more frequent re-examination may be imperative to maintain diagnostic sensitivity with steeply increasing incidences in the oldest old.[20] In these individuals, linkage to health care records is helpful, but not sufficient by itself in light of notorious under-investigation and -diagnosis in this age group. Finally, for trends analysis, a potential disadvantage of the linkage is the closer correlation with health care policy. Higher detection rates could counterbalance a decline in the incidence of disease with increased attention for dementia over time,[193] and this might also have led to underestimation of incidence trends in the Rotterdam Study.

Competing risks

With advancing age, numerous hazards fight for priority to cause death and disability. Although we mostly think of death as a rather unequivocal event, the counterfactual world of the epidemiologist begs to differ. The interplay of diseases, which occurs particularly at old age, becomes a potential threat for the validity of a study when interest lies in one specific disease outcome. Dementia mostly manifests late in life, at a time by which many other diseases may already have had a shot at reducing one's lifespan, thereby precluding the development of dementia. As many risk factors are shared between diseases, the competing event of death will more likely affect those who are also at highest risk of the disease of interest, thereby hampering its proper study.

The subject of competing risks dates back as far as the 18th century, when Swiss physician and mathematician Daniel Bernoulli studied the possible consequences of eradication of smallpox on (cause-specific) mortality rates (Figure 14).[194] His calculations were arguably the first mathematical model used in epidemiology, which might not have happened, had Daniel's somewhat envious father – and himself renowned mathematician – Johann not asked his son to study medicine rather than mathematics, to which Daniel reportedly agreed only if his father would tutor him in mathematics privately. The problem of estimating failure probabilities in light of (elimination of) competing risks gained increasing attention in the second half of the 20th century,[195,196] culminating in the introduction of the nowadays familiar subdistribution hazards model by Jason Fine and Robert Gray in 1999.[197] The application and interpretation of these models, however, remain a challenge in clinical research.[198]

While competing risk modelling, for example with the subdistribution hazard of Fine and Gray's models, can be valuable in prognostic studies, they are less appropriate for determining aetiological associations in the presence of strong competing risks.[199,200] The fundamental issue with competing risk is that one of the main assumptions for censoring, independence of reasons for censoring, is no longer met. For estimating prognosis, ignoring the fact that death precludes development of an illness overestimates an individual's risk, and one would therefore intuitively want to keep a person in the risk set after occurrence of this competing event. Conversely, in aetiological studies, the primary interest lies in determining the (relative) risk of disease in those who are still at risk of the disease at a certain time-point. These cause-specific hazards can be obtained from a Cox proportional hazards model,[201] in which individuals are censored at time of (competing) event, and which importantly does not require independence of censoring to produce valid risk estimates.[199,200]

For these reasons, I have used subdistribution hazard models notably in Chapters 2.2 and 5.4 to compute absolute risks, but cause-specific hazards throughout other aetiological studies requiring survival analysis. Of note, neither form of modelling addresses potential bias caused by competing events 'masking' the impact of the risk factor on the phenotype of interest. As most exposures examined in this dissertation are also associated with increased mortality, this will generally have led to underestimation of the true causal association. Novel analytical methods are warranted to account for this bias, or alternatively, application of markers sensitive to early neurodegenerative changes may in part circumvent the issue of the competing risk of death.

TABLE I.

AGES par années.	Survivans selon M. Halley.	N'ayant pas eu la pet. vérole.	Ayant eu la pet. vérol.	Prenant la pet. vérole pendant ch. année.	MORTS de la pet. vérole pendant chaq. ann.	SOMME des morts de la pet. vérole.	MORTS par d'autres maladies pend. chaq. année.
0	1300	1300	0				
1	1000	896	104	137	17,1	17,1	283
2	855	685	170	99	12,4	29,5	133
3	798	571	227	78	9,7	39,2	47
4	760	485	275	66	8,3	47,5	30
5	732	416	316	56	7,0	54,5	21
6	710	359	351	48	6,0	60,5	16
7	692	311	381	42	5,2	65,7	12,8
8	680	272	408	36	4,5	70,2	7,5
9	670	237	433	32	4,0	74,2	6
10	661	208	453	28	3,5	77,7	5,5
11	653	182	471	24,4	3,0	80,7	5
12	646	160	486	21,4	2,7	83,4	4,3
13	640	140	500	18,7	2,3	85,7	3,7
14	634	123	511	16,6	2,1	87,8	3,9
15	628	108	520	14,4	1,8	89,6	4,2
16	622	94	528	12,6	1,6	91,2	4,4
17	616	83	533	11,0	1,4	92,6	4,6
18	610	72	538	9,7	1,2	93,8	4,8
19	604	63	541	8,4	1,0	94,8	5
20	598	56	542	7,4	0,9	95,7	5,1
21	592	48,5	543	6,5	0,8	96,5	5,2
22	586	42,5	543	5,6	0,7	97,2	5,3
23	579	37	542	5,0	0,6	97,8	6,4
24	572	32,4	540	4,4	0,5	98,3	6,5

Figure 14. The table with Bernoulli's calculations, based on the life table figures (i.e. the first two columns) presented earlier by Halley.[202] Data originated from the city of Breslau in Austrian Silesia (presently Wroclaw, Poland). At the time, Breslau was considered representative of the natural evolution of a human population given its minimal migration; much alike the choice for the Ommoord area as the epicentre of the Rotterdam Study nearly 300 years later.

Residual confounding and overadjustment

Confounding, from the Latin *confundere* ("pour together") is "confusion, or mixing, of effects; the effect of the exposure is mixed together with the effect of another variable, leading to bias,"[203] considered one of the major threats to the validity of observational study. Once again, I have been fortunate that the design of the Rotterdam Study allowed for adjustment of many potential confounders. Nevertheless, I cannot exclude the possibility of residual confounding, either by exposures unadjusted for, or incompletely captured by the definition at hand. It is a remarkable fact that despite the profound share of vascular pathology in the aetiology of dementia, adjustment for traditional cardiovascular risk factor left effect estimates virtually unchanged across analyses in this dissertation. Either the determinant of interest was – somewhat unlikely – a perfect intermediate of their association with dementia, there was truly no association between confounder and exposure or outcome in the yielded data, or the effect of the potential confounder on either

exposure or outcome was insufficiently captured by the definition used in my analyses. The latter could particularly arise for risk factors with effects arising after prolonged exposure over years if not decades. For example, the associations of obesity with dementia reverses with advancing age,[204] and adjustment for body mass index may in elderly participants not fully capture this effect. The same may apply to hypertension. With longer follow-up and historical measurements of participants available, it could be worthwhile investigating whether mid-life effects of risk factors on dementia incidence may be partly accountable for any residual confounding in late-life study. A priori, the impact of such residual confounding is hard to estimate, but the degree of confounding needed to negate any observed effect may be more easily assessed using the recently coined *E-value*.[205] The *E-value* allows for sensitivity analysis regarding unmeasured confounding without any assumptions about the underlying structure of the confounder, and provides a value for the strength of the exposure-confounder and confounder-outcome relationships needed to dilute the effect estimate of interest (or its lower confidence bound) to the null. Its calculation is rather straightforward,[205] using the risk ratio (RR): $E = RR + \sqrt{\{RR * (RR - 1)\}}$. Applying this formula to the association between anaemia and incident dementia in Chapter 3.4 provided some insights in the degree of confounding needed (in this case a RR of 2.0 for both the exposure-confounder and confounder-outcome association) to account for an association with a RR of 1.4 in the main analysis. This technique, if it were to become common practice, could facilitate assessment of observational evidence, and the recently proposed straightforward application is a huge push in the right direction. Controlling for confounding, however, one can feel trapped between a rock and a hard place. While accounting for potential confounding, a danger lures on the other side: unnecessary adjustment or over-adjustment. Whether by reduction in precision due to control for a variable that does not affect bias, or by control for an intermediate variable,[206] these may undermine conclusions about the association under investigation. In my analyses, I have aimed to carefully select covariates on the basis of existing mechanistic knowledge of the association of interest,[207] rather than by empirical testing for significance of individual covariates (many smaller, statistically non-significant effects can altogether create a meaningful bias). Nevertheless, mechanisms are often not all accounted for, or can get intertwined in complex pathophysiology, which may at times have led to unnecessary adjustment.

IMPLICATIONS AND FUTURE PERSPECTIVES

Der Wahrheit ist allerzeit nur ein kurzes Siegesfest beschieden, zwischen den beiden langen Zeiträumen, wo sie als Paradox verdammt und als Trivial gering geschätzt wird. – Arthur Schopenhauer, *Die Welt als Wille und Vorstellung (1818)*

Of this thesis, three broad implications may be taken forward. First, the potential for prevention of dementia, in light of compression of morbidity in late-life, deserves advocacy to both policy makers and the general public. Preventive interventions with small effects at the individual level, and relatively minor postponements in the onset of dementia could have a major impact on the burden of disease at the population level. Continuous monitoring of disease occurrence is thereby crucial to detect changes in public health, and observe the effects of our preventive undertakings and other contemporary trends. At the same time, health care systems need to prepare for this growing burden of disease by allocation of resources to prevention, diagnosis, and care. Already, 87% of current dementia care costs are incurred in high-income countries,[208] where cost for dementia diagnosis in specialist care outweigh those in primary care by a factor of 10.[208] As the burden of dementia grows across the globe, the largest increase will occur in low- and middle-income countries, such that by 2050 roughly two thirds of people with dementia will live in these regions. It shows above all that worldwide availability and accessibility of diagnostics, preventive measures, and feature disease-modifying agents will be vital to truly control the dementia epidemic.

In order to prevent disease, it is vital to understand the aetiology, and have identified sufficient risk factors and indicators. Regarding preventive treatments, I believe that there is sufficient cause to pursue advanced insight in cerebral haemodynamic changes as a risk factor for cognitive decline. Well-designed longitudinal studies are needed to link neurovascular function to amyloid pathology, other markers of neurodegeneration, and dementia. Such an approach, acknowledging the entire spectrum of highly prevalent brain pathology in the elderly, may at last provide a paradigm that survives well outside highly selective specialist environments. A two-way interaction between *bench and bedside* is thereby likely to benefit translation of both into meaningful preventive strategies at a population level. This opens up new possibilities to population-based cohorts, like the Rotterdam Study. It is my belief that the traditional trade-off between the numbers needed to observe sufficient disease outcomes versus the desired and feasible detail in mapping various phenotypes will have to shift towards the latter. Notwithstanding the value of concurrent measurements linking various organ systems and disease characteristics, I believe that driven by the need for the understanding of biological mechanisms, and sped up by an ever increasing data availability,[209,210] a next level of detail in the phenotyping of cohorts of several thousands of participants is necessary to yield their potential, and return the substantial investment by society. The tools we have in our hands to achieve this are promising. Broad availability of advanced imaging techniques, induced pluripotent stem cells to deliver *organs-on-a-chip*, genetic modification using CRISPR-CAS. In addition, much can

still be learned from yielding existing methods and data in a more fruitful and imaginative way. The physiological effects of widely prescribed medication, notably of the antihypertensive kind, on cerebrovascular resistance and reactivity. The use transcranial Doppler, near-infrared spectroscopy, or perfusion MRI to map vivo response to challenges on brain perfusion. Ways to improve methodological rigour by transferring knowledge of epidemiology, and applying no more than its fundamental principles throughout science. Possibilities to apply and investigate existing vascular care for benefit on cognition as well as survival and (recurrence) of vascular events. These are mere examples. Perhaps in the not so distant future, we shall see cognitive wellbeing integrated in care of patients with heart disease or stroke, and vice versa, at multidisciplinary outpatient clinics. Perhaps not too far from now, health and disease will (once again) be seen in an organ transcending manner, better preserved than restored.

CONCLUDING REMARKS

A medical library search for dementia-related publications over the last year yields over 12,500 results, equalling about 35 studies per day. This number incites the rather unsettling thought that nobody is aware of the full literature on dementia or Alzheimer's disease. How many of the forgotten findings should have been remembered? Which of today's writings will be remembered in 100 years? Perhaps, in the final sentences of this thesis, it is prudent to bring to stage Alois Alzheimer. In addition to amyloid accumulation, Alzheimer noticed lipid deposition in his pathological specimens as he wrote in 1906: "*Die Glia hat reichlich Fasern gebildet, daneben zeigen viele Gliazellen große Fettsäcke. Ein Infiltration der Gefäße fehlt vollig. Dagegen sieht man an den Endothelien ucherungserscheinungen, stellenweise auch eine Gefäßneubildung.*"[211] These findings – magnificently illustrated by Alzheimer and his Italian pupil Gaetano Perusini[212] – have been largely ignored for many years. Now that we delve into the pathophysiology of *APOE*, we can perhaps begin to grasp the full spectrum of pathology described already more than a century ago. It exemplifies that it is possible, perhaps even likely, that the most evident aetiological factors in dementia are overlooked in this dissertation. As with the largely ignored observations of lipid accumulation and vascular proliferation by Alzheimer, the eyes do not see what the mind does not know. I do hope, however, that this thesis shall prove one tiny step forward, and that quite a few small steps from now, we shall live to remember how the full potential for prevention of this dreadful disease was achieved.

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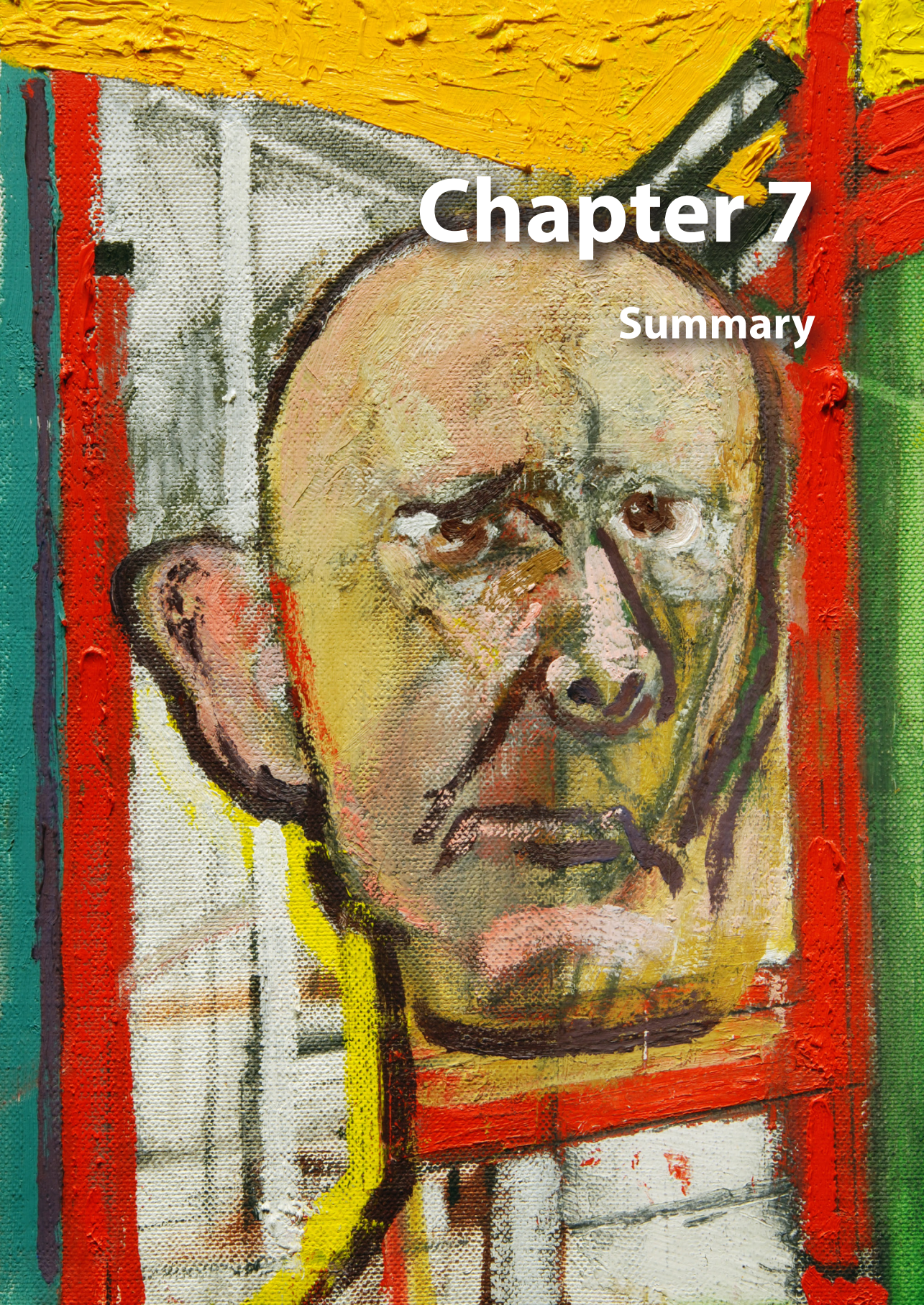

Chapter 7

Summary

SUMMARY

Recent years have seen a surge in dementia research, brought on by an increasing awareness of its present and pending consequences for public health. This focus on dementia has revealed a multifaceted surface, shaped by decades of exposure to a variety of risk factors, but what remains at its core is yet unknown. In this thesis, I examine the roughness of its edges, before scratching the surface to examine what lies underneath.

In the first part of this thesis, I demonstrate the enormity of the burden of dementia, which affects 1 in 3 women, and 1 in 5 men during the course of their lifetime (**Chapter 2.2**). These high risks are also reflected in **Chapter 2.1** in lifetime spent with dementia, which increases from 6% of remaining life years at age 65 to 42% at age 95 for women, and from 4% to 35% at the same ages for men. This high burden of disease in potentially highly amendable by preventive interventions at the population level, supported by decreases of about 30-50% following preventive scenarios that delay disease onset by 1 to 3 years (**Chapters 2.1 & 2.2**). The preventive potential is corroborated by declines in the age-specific incidence of dementia over the past decades, described in **Chapter 2.3**. Although insufficient to offset the growing burden of disease due to ageing of populations, these offer reason for cautious optimism, and encouragement for development and wider implementation of preventive strategies based on the causes of the declining incidence trends.

Focussing the loupe on cerebral blood supply, I show in **Chapter 3.1** that low cerebral perfusion predisposes to the development of dementia during on average 7 years of follow-up, in particular in the presence of cerebral small-vessel disease or hypertension. A causal relationship is supported by effects of carotid artery stenosis on brain atrophy in **Chapter 3.5**. Transient episodes of cerebral hypoxia due to impairment of cerebral autoregulatory mechanisms and oxygenation may contribute to this association, as evidenced by increases in dementia risk with orthostatic blood pressure in **Chapter 3.2**, impaired cerebrovascular reactivity in **Chapter 3.3**, and disturbed haemoglobin homeostasis in **Chapter 3.4**.

A systematic review of the literature in **Chapter 4.1** establishes that coronary heart disease and in particular heart failure are associated with the risk of developing dementia. This could reflect consequences of diminished (inotropic regulation of) cerebral blood flow, but also thromboembolic complications (for example involving Von Willebrand factor and ADAMTS13, **Chapter 4.4**), direct effects of natriuretic peptides, or shared aetiological mechanisms with a pro-inflammatory state, or relating to (vascular) amyloid-β40 in **Chapter 4.3**. In contrast to coronary heart disease and heart failure, aortic valve calcification without

haemodynamically significant stenosis does not appear to contribute to this risk increase, based on a 10-year follow-up study in **Chapter 4.2**.

In **Chapter 5**, I shift focus to the heritability of dementia. Effects of the most important common genetic risk factor for dementia, the Apolipoprotein E gene (*APOE*), on mortality in **Chapter 5.1** illustrate that investigation of *APOE* in wider health and disease could prove useful in understanding biological mechanisms underlying dementia. Applying *APOE* carrier status, along with other identified common genetic risk variants of dementia to risk prediction of dementia in **Chapter 5.4**, I show that yielding genetic information allows risk stratification into low- and high-risk in the community, with absolute risks by age 85 ranging from 4% in the lowest risk category to 63% in the highest risk group, translating into a roughly 20-year difference in age at onset of dementia. These risks may be further refined by taking a specific family history (**Chapters 5.3 & 5.4**). A comparison of four cohorts in **Chapter 5.2** shows that for application of these absolute risks to trial design and individual risk prediction, it is vital to account for the source population and individual characteristics. Further refinement of genetic screening may come from assessment of peripheral levels of gene products, as exemplified by the additional predictive value of serum levels of apolipoprotein E above and beyond the *APOE* genotype in **Chapter 5.5**.

To conclude, I discuss in **Chapter 6** these main findings in light of published literature, and provide methodological considerations and recommendations for future research. A future in which identification of the causes of declining dementia incidence can serve as the foundation for preventive strategies. In which additional targets for prevention can be provided by insight in the physiological mechanisms than maintain cerebral perfusion and oxygenation, and the effects of hypoxia on neurons and glial cells if these mechanisms falter. A future in which the substantial overlap of dementia not only with stroke, but also with heart disease, sees integration of (vascular) care across medical specialties restored. And a future in which genetic factors are applied to aetiological study, as well as precise prognosis and targeted intervention. For the coming years, with the exciting potential of technological advances, and further integration of observational and translational research in light of established core principles of epidemiology, we are well set up to strive and take dementia into the realm of forgotten diseases.

SAMENVATTING

Onderzoek naar dementie heeft de afgelopen jaren een grote impuls gekregen, gedreven door de onderkenning van haar huidige en toekomstige consequenties voor de publieke gezondheid. Deze gespitste blik op dementie heeft een veelzijdig ziektebeeld onthuld, gevormd door decennialange blootstelling aan een variëteit aan risicofactoren. Desalniettemin blijft de aetiologie van dementie in de kern vooralsnog onbekend. In dit proefschrift beschouw ik de ziektelast van dementie op populatieniveau, alvorens over te gaan tot onderzoek van etiologische en mogelijk predictieve factoren.

In het eerste deel van dit proefschrift breng ik de enorme omvang van de ziektelast van dementie in kaart. Dementie treft 1 uit 3 vrouwen, en 1 uit 5 mannen gedurende hun leven (**Hoofdstuk 2.2**). Deze hoge risico's komen ook tot uiting in de levensjaren doorgebracht met dementie, welke voor vrouwen toeneemt van 6% van de levensverwachting op 65-jarige leeftijd tot 42% op de leeftijd van 95, zoals beschreven in **Hoofdstuk 2.1**. Voor mannen betreffen deze percentages 4 en 35%. Deze hoge ziektelast is mogelijk te verminderen door preventieve interventies op bevolkingsniveau, hetgeen wordt onderschreven door reducties in risico en levensjaren met dementie van 30-50% in scenario's waarbij preventieve interventies de ziekte 1 tot 3 jaar uitstellen (**Hoofdstukken 2.1 & 2.2**). De potentie van preventie wordt verder onderschreven door een afname in de leeftijdsspecifieke incidentie van dementie over de afgelopen decennia in Europa en Noord-Amerika, beschreven in **Hoofdstuk 2.3**. Hoewel deze afnames onvoldoende zijn om de groeiende ziektelast door veroudering van de populatie op te vangen, bieden zij wel reden voor voorzichtig optimisme, en aanzet tot ontwikkeling en wijdere implementatie van preventieve strategieën gebaseerd op de oorzaken van de geobserveerde trends in de incidentie.

De focus verleggend naar de bloedvoorziening van de hersenen als mogelijke etiologische factor in dementie, laat ik in **Hoofdstuk 3.1** zien dat lage doorbloeding van de hersenen een groter risico op dementie met zich meebrengt gedurende gemiddeld 7 jaar dat deelnemers werden gevolgd. Dit is in het bijzonder het geval wanneer reeds sprake is van hypertensie of schade aan de kleine bloedvaten in de hersenen. Causaliteit in deze associatie wordt ondersteund door effecten van stenosering van de arteria carotis interna op cerebrale atrofie in **Hoofdstuk 3.5**. Korte episodes van cerebrale hypoxie, te wijten aan verstoorde autoregulatie en oxygenatie, kunnen verder bijdragen aan de associatie tussen hypoperfusie en dementie. Dit laat ik zien aan de hand van verhoogd risico op dementie met orthostatische bloeddrukdalingen in **Hoofdstuk 3.2**, met verstoorde cerebrovasculaire reactiviteit in **Hoofdstuk 3.3**, en verstoorde hemoglobine homeostase in **Hoofdstuk 3.4**.

Een systematische beschouwing van de literatuur in **Hoofdstuk 4.1** stelt vast dat coronair vaatlijden en in het bijzonder hartfalen geassocieerd zijn met een hoger risico op het ontwikkelen van dementie. Dit kan het gevolg zijn van (inotropische) verstoringen van de cerebrale perfusie, maar kan ook resulteren uit thrombo-embolische complicaties (mogelijk in relatie tot Von Willebrand factor en ADAMTS13, **Hoofdstuk 4.4**), directe effecten van natriuretische peptides, of gedeelde etiologische mechanismen bij een pro-inflammatoire status, of in relatie tot (vasculair) amyloid-β40 in **Hoofdstuk 4.3**. In tegenstelling tot coronair vaatlijden en hartfalen, lijkt calcificatie van de aortaklep van het hart zonder hemodynamisch significante stenosering niet bij te dragen aan deze risicoverhoging, gebaseerd op een studie met 10 jaar follow-up in **Hoofdstuk 4.2**.

In **Hoofdstuk 5** verleg ik de focus naar de erfelijkheid van dementie. Effecten van de verreweg belangrijkste veelvoorkomende genetische risicofactor voor dementie, het Apolipoproteine E gen (*APOE*), op mortaliteit in **Hoofdstuk 5.1** illustreren dat onderzoek van APOE buiten het veld van dementie kan bijdragen aan inzicht in biologische mechanismen die tot dementie leiden. Het toepassen van *APOE* dragerschap, tezamen met andere bekende frequent voorkomende genetische risicovarianten, in de predictie van dementie staat toe om laag- en hoog-risico groepen in de bevolking te identificeren (**Hoofdstuk 5.4**), waarbij absolute risico's tot de leeftijd van 85 jaar uiteen lopen van 4% in de laagste risicocategorie tot 63% in de hoogste risicocategorie, hetgeen zich vertaalt in een verschil van 20 jaar in leeftijd bij diagnose. Deze risico's kunnen verder worden gespecificeerd met behulp van een specifieke familieanamnese (**Hoofdstukken 5.3 & 5.4**). Een vergelijking van vier cohorten in **Hoofdstuk 5.2** laat echter zien dat het voor individuele risicovoorspelling en toepassing van deze absolute risico's in klinische trials cruciaal is om de onderliggende populatie en persoonlijke karakteristieken mee te wegen. Verdere verbetering van genetische risicopredictie kan mogelijk komen uit meting van perifere genproducten, zoals serum Apolipoproteine E in **Hoofdstuk 5.5**.

Tot slot bespreek ik in **Hoofdstuk 6** al bovenstaande bevindingen in het licht van gE-publiceerde literatuur, waarbij ik ruim aandacht geef aan methodologische factoren, en aanbevelingen doe voor toekomstig onderzoek. In de toekomst zie ik een belangrijke rol voor het identificeren van oorzaken van afnames in de incidentie van dementie als leidraad voor preventieve strategieën. Aanvullende doelwitten voor preventieve interventies kunnen voortkomen uit inzicht in fysiologische mechanismen die cerebrale doorbloeding en oxygenatie waarborgen, en uit de effecten van hypoxie op neuronen en gliale cellen als deze mechanismen falen. In de toekomst zie in toenemende mate aandacht voor de overlap van dementie met niet alleen beroertes, maar ook hartziekte, waarbij integratie van (vasculaire) geneeskunde tussen diverse medische specialismen in ere wordt hersteld. Genetische

risicofactoren zullen hierbij in toenemende mate worden toegepast in etiologische studies, alsook voor prognose en gerichte interventie. Met een veelbelovend arsenaal aan technologische ontwikkelingen, en verdere integratie van observationeel en translationeel onderzoek op de grondslagen van de epidemiologie hebben wij de komende jaren alle gereedschap in handen om ernaar te streven dementie te verwijzen naar het land van vergeten ziektes.

EPILOGUE

"*It is not sufficient to examine. It is also necessary to observe and reflect. And we should make these observations our own where the heart is concerned, as well as in an intellectual sense. Only then will they surrender their secrets to us, for enthusiasm heightens and refines our perception. As with the lover who discovers new perfections every day in the woman he adores, he who studies an object with an endless sense of pleasure finally discerns interesting details and unusual properties that escape the thoughtless attention of those who work in a routine way*" (Santiago Ramón y Cajal – *Reglas y Consejos sobre Investigación Cientifica,* 1899).

Every morning when I strolled through the Museum Park, and saw the ivory-white research tower emerge behind the trees, I felt fortunate to dwell within the world of academia. It is a privilege to see the playground of one's own curiosity merge with the wider accumulation of knowledge; to partake in progress, generally slow and often imperfect, but progress nonetheless towards a healthier world. The path of the young researcher in this world is marked by a growing awareness of what is yet unexplained. I have often felt astonished by the vast number of outstanding questions within one relatively small area in the realm of medicine, enough to keep my mind occupied for years to come.

These questions cannot be answered without the integration of observation and experiment. The experimental scientist has the advantage of avoiding certain biases that threaten conclusions about cause and effect in observational studies. Yet, to suppose that observation is inherently incapable of answering on the question of causation has always seemed to me an impudent attack on human intellect. The biases that lure in observation should nevertheless not be taken lightly. This is not an easy challenge, as navigating amidst Scylla and Charybdis, the avoidance of spurious claims of causality may easily leave one trapped in a strict methodological dogma with very little empirical implication. The only solution I see for this is to apply methodological expertise on a firm foundation of knowledge in physiology. A certain degree of pragmatism on the side of the epidemiologist may thereby serve us well in effectively enriching clinical research practice with methodological rigour.

The past four years would not have been nearly as exciting without many of the people I encountered along the way. More than in any other field of research, obtaining a doctoral degree in medicine is a team effort, and more than in any other medical research undertaking, this is the case within the Rotterdam Study. Quoting the famous words of Bernard de Chartres, if I have seen further it is only because I have been standing on the shoulders of giants. I am indebted to all – research team and study participants alike –

whose goodwill over the past 28 years has provided me with the opportunity to complete this thesis. Frequent visits to Ommoord were a pleasant reminder of this, and the interaction with patients and participants an indispensable part of my training as a clinical epidemiologist.

This adventure would not have commenced, nor come to a successful ending without the confidence and support of Professors Arfan Ikram and Peter Koudstaal. It is thanks to your skill, experience, and encouragement that I have been able to develop into an independent researcher. Professor Albert Hofman, I am most grateful for having had the opportunity to study under your auspices in the rich intellectual environment of the Harvard School of Public Health. At the cradle of my professional existence, I further distinguish Professors Jan van Gijn and Peter Rothwell. Many a day I gratefully acknowledge your belief in the potential of a young man with no prestigious official credentials. I can only wish to develop into such an inspiring mentor to others, as each of you has been to me. Likewise, many colleagues, notably of the Heart Brain Connection collaboration, the Alzheimer Cohorts Consortium, and the CHARGE consortium have been a huge source of inspiration over the past years. Co-investigators of various studies within this thesis I owe my thanks for bettering my reasoning and writing. I would also like to thank all members of the reading committee and opposition, Professors Elly Hol, Hugh Markus, Francesco Mattace Raso, René Melis, Sudha Seshadri, and Meike Vernooij for their precious time devoted to the appraisal of this work.

For encouragement, balance, focus, and timely distraction, I have to thank many friends, who from the proximity of the departmental 28th floor to distant parts of the world were always close at heart. Any personal note is best handwritten, but as common ground these will have my gratitude for the joy, affection, and way our friendships feed mutual development. Whether bonds grow stronger over time, or at times fade into memory, each leaves a permanent mark, which I treasure and consider invaluable.

Tot slot, mijn lieve ouders. Alles wat ik heb bereikt, is dankzij de mogelijkheden die jullie mij hebben gegeven. Mijn geluk daarmee is niet te beschrijven. Dit werk is een direct gevolg van de verantwoordelijkheid die jullie mij meegaven om mijn talenten optimaal te benutten. Ik zal altijd blijven streven mij door deze les te laten leiden.

Frank J. Wolters, June 2018

Appendices

PHD PORTFOLIO

Name PhD student:	F.J. Wolters
Research school:	Netherlands Institute for Health Sciences (NIHES)
Erasmus MC department:	Epidemiology
PhD period:	May 2014 – February 2018
Supervisors:	Professors M.A. Ikram and P.J. Koudstaal

Activity	Year	ECTS*
1. PhD training		
General courses		
Master of Science in Clinical Epidemiology (NIHES)	2014-2016	70
Vascular biology (Dutch Heart Foundation)	2014	1.5
Scientific integrity (Erasmus MC)	2015	0.3
Neuro-epiomics (Boston University)	2016	1.5
Cambridge Dementia Course (Cambridge, UK)	2017	1.0
International conferences		
International Atherosclerosis Society International Symposium (Amsterdam, NL)	2014	1.0
Alzheimer's Association International Conference (Washington DC, USA)	2015	2.0
American Academy of Neurology Annual Meeting (Vancouver, Canada)	2016	3.0
Alzheimer's Association International Conference (Toronto, Canada)	2016	2.0
International Society of Vascular Behavioural and Cognitive Disorders International Meeting (Amsterdam, NL)	2016	2.0
Alzheimer's Association International Conference (London, UK)	2017	1.0
European Academy of Neurology Annual Meeting (Amsterdam, NL)	2017	1.0
Translational Neuroscience Network (TN2) Conference (Amsterdam, NL)	2017	1.0
Workshop, seminars, and symposia		
Biannual Heart Brain Connection collaborative research group meeting	2014-2017	5.0
Biannual Alzheimer Cohorts Consortium workshop	2016-2017	3.0
Journal club (Epidemiology)	2014-2017	2.0
Departmental seminars (Epidemiology)	2014-2017	2.0
Departmental seminars (Neurology)	2014-2017	2.0
Research visits		
Harvard T.H. Chan School of Public Health	2016	

2. Teaching activities		
Teaching assistance		
Principles of research in medicine and epidemiology (NIHES)	2015	0.5
Biostatistics I (NIHES)	2015	0.5
Clinical trials (Medicine, Erasmus MC)	2016-2017	1.0
Fundamentals of epidemiology (Harvard School of Public Health, Boston, USA)	2016	4.0
Invited lectures		
General practitioner in-service training about risk factors for dementia (LAEGO)	2016	0.2
Lay audience talk about prevalence and incidence of dementia (Deltaplan Dementie)	2016	0.2
Project supervision		
Master's project: Helicobacter Pylori infection and risk of dementia	2016	1.5
High school graduation project: Public perception of risk factors for Alzheimer's disease	2016	0.5
Junior Med School: Arterial blood supply to the brain – does size matter?	2017	1.0
High school graduation project: Shared genetic susceptibility to cardiovascular risk factors and cerebral small-vessel disease and neurodegeneration on brain MRI	2017	1.0
3. Other activities		
Peer-review	2014-2018	2.5
Scan appraisal for incidental findings in population imaging	2015-2017	2.0
National Coordinator of the Dutch Surveillance Centre for Prion Disease	2015-2018	4.0
Student panel for the Epidemiology Master's degree program (NIHES)	2014-2016	2.0
Data management user panel (Epidemiology)	2015-2017	1.0

* 1 ECTS (European Credit Transfer System) equals a workload of 28 hours

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Wolters FJ, Li L, Gutnikov SA, Mehta Z, Rothwell PM – *Medical attention seeking after transient ischemic attack and minor stroke before and after the UK Face, Arm, Speech, Time (FAST) public education campaign: Results from the Oxford Vascular Study*. JAMA Neurol. 2018; E-pub ahead of print.

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Wolters FJ, De Bruijn RF, Ikram MA – *Potential association between atrial fibrillation and dementia–reply [Letter]*. JAMA Neurol. 2016;73(5):607-8.

Wolters FJ, Koudstaal PJ, Hofman A, van Duijn CM, Ikram MA. *Serum apolipoprotein E is associated with long-term risk of Alzheimer's disease: the Rotterdam Study.* Neurosci Lett. 2016;617:139-42.

Wolters FJ, De Bruijn RF, Hofman A, Koudstaal PJ, Ikram MA – *Cerebral vasoreactivity, apolipoprotein E, and the risk of dementia: a population-based study*. Arterioscler Thromb Vasc Biol. 2016;36(1):204-210.

Wolters FJ, Ikram MA – *Delayed-start analysis for demonstrating disease modification of solanezumab [Letter].* Alzheimers Dement (N Y). 2015;1(3):196-197.

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ABOUT THE AUTHOR

Franciscus (Frank) Johannes Wolters was born on 16th August 1985 in Zwolle, the Netherlands. He grew up in a loving family in the nearby township of Raalte, and following his graduation from the local gymnasium moved to Utrecht to study Medicine. After obtaining his medical degree from Utrecht University in 2011, he pursued his interest in clinical neurology as a registrar at the department of Neurology of the University Medical Centre Utrecht, before taking up a clinical research fellowship at the University of Oxford (UK) a year later. Oxford proved a felicitous next step after many years in picturesque Utrecht, inciting interest in preventive medicine by studying the prevention of early recurrent stroke after transient ischaemic attack and minor stroke in the Oxfordshire community. In spring of 2014, a PhD scholarship within the Rotterdam Study led Frank back to the Netherlands, thereby shifting his research interest from clinical cerebrovascular disease to vascular cognitive impairment and dementia, as witnessed by this thesis. In Rotterdam, Frank obtained a Master's degree in Clinical Epidemiology at the Netherlands Institute of Health Sciences. Eager to learn in different academic environments, he performed part of the work for this thesis at the Harvard T.H. Chan School of Public Health in Boston (MA, USA), supported by a personal fellowship of the Dutch Alzheimer Foundation. In the ever-decreasing amount of spare time, Frank appreciates non-scientific literature, artwork exhibitions, and wine-facilitated discussion about the essence of life. Following completion of this thesis, he has resumed clinical training at the department of Neurology within the Erasmus Medical Centre, and will continue to explore means for prevention of cognitive decline with support of a Young Talent Program grant of the Dutch Heart Foundation.