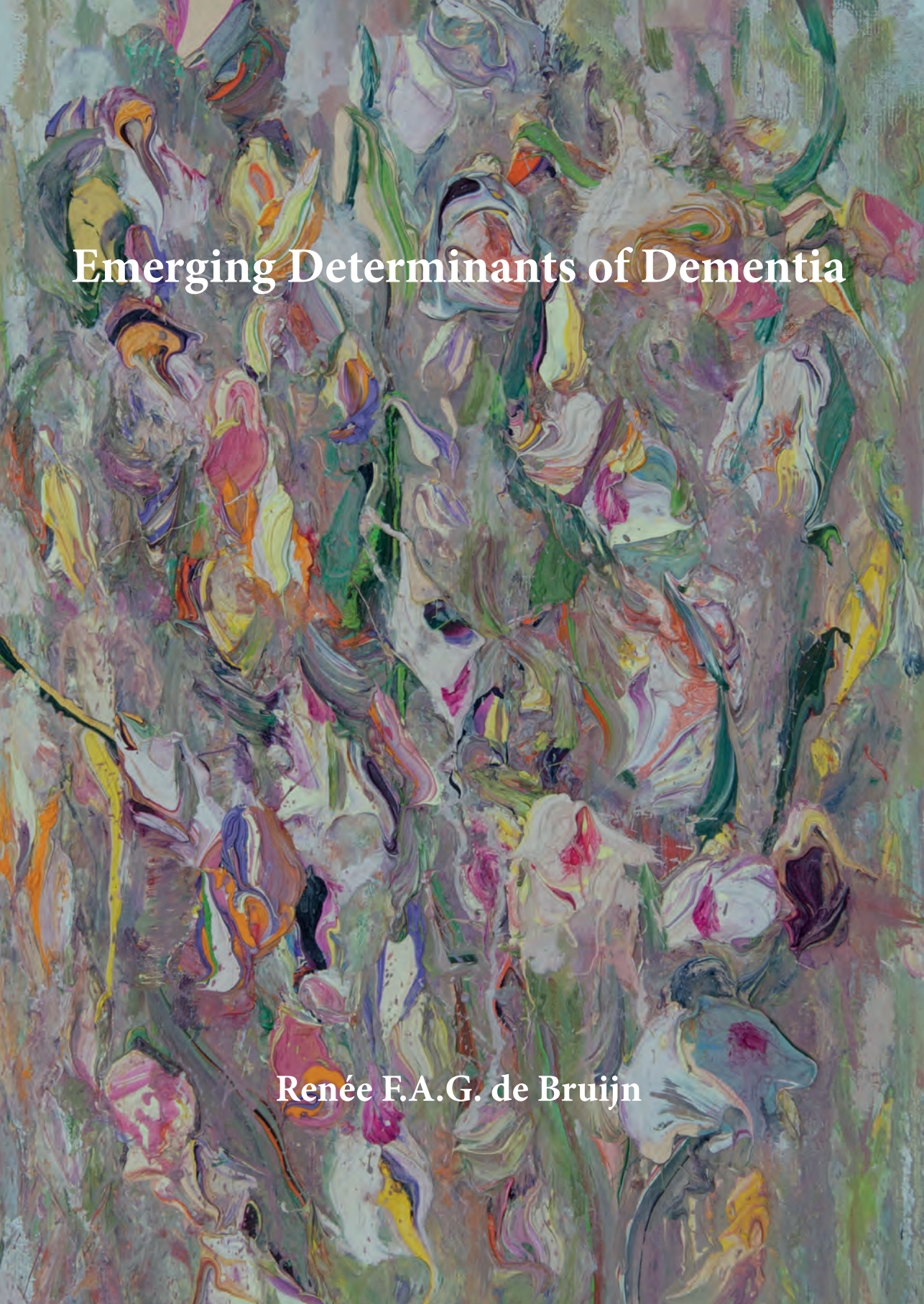

Emerging Determinants of Dementia

Renée F.A.G. de Bruijn

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Manuscripts based on the studies described this thesis

1. de Bruijn RFAG, Ikram MA. Cardiovascular risk factors and future risk of Alzheimer's disease. *BMC Medicine* 2014; 12:130
2. de Bruijn RFAG, Bos MJ, Portegies MLP, Hofman A, Franco OH, Koudstaal PJ, Ikram MA. The potential for prevention of dementia across two decades: the Rotterdam Study. *Submitted*
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Chapter 1

General introduction

Dementia is one of the most common and devastating diseases in the elderly. About 40 million people worldwide are suffering from the disease, and this number is expected to rapidly increase in the coming decades due to aging of the population.[1] Dementia is characterized by loss of neuronal tissue in the brain leading to cognitive impairment severe enough to interfere with daily life.[2] As the disease progresses, cognitive abilities further decline and eventually patients become completely dependent on care. The etiology of dementia is still largely unknown and there is no effective curative therapy. Against this background, dementia is increasingly being recognized as a major health problem and much research is being undertaken to unravel its etiology, find an effective treatment, and explore the potential for prevention of dementia.[1]

There are many subtypes of dementia, of which Alzheimer disease and vascular dementia are the most common. For many years, it has been suggested that these dementia subtypes have distinct etiologic features: amyloid plaques and neurofibrillary tangles in Alzheimer disease and vascular pathology in vascular dementia.[3,4] However, accumulating evidence suggests that vascular pathology not only plays an important role in vascular dementia, but also in Alzheimer disease.[5-7] Accordingly, several cardiovascular risk factors, such as hypertension, diabetes, and overweight have been identified as risk factors of dementia, including Alzheimer disease.[8] As most of these factors are modifiable, dementia could potentially be prevented or postponed by optimal treatment of these risk factors.[8,9] Neuropathologic changes related to dementia gradually accumulate over years before clinical symptoms occur.[10] By the time dementia is diagnosed, the disease has already caused irreversible damage. Preventive options should therefore be implemented timely, that is, many years before dementia is clinically diagnosed. This underlines the need to identify early features of the disease, such as mild cognitive impairment (MCI). MCI is regarded as an intermediate phase between normal aging and dementia, during which people do have cognitive problems but not severe enough to interfere with daily functioning.[11] Although MCI has been widely investigated, findings across studies vary largely due to methodological differences.[12]

Besides exploring the impact of known risk factors, there is an ongoing quest for novel risk factors of dementia, such as metabolic and behavioral factors. As described above, dementia has a long preclinical phase, during which neuropathology slowly accumulates. Therefore, it is essential to examine potential risk factors of dementia in prospective studies with long follow-up periods. Otherwise, it would be impossible to disentangle whether a factor is causal or rather an early symptom of disease. Furthermore, potential risk factors need to be investigated in population-based settings to overcome methodological limitations of hospital-based studies.

The aim of this thesis was two-fold. On the one hand, I wanted to explore the impact of known risk factors on dementia and on the other hand, I aimed to identify novel risk factors. All research described in this thesis, is embedded within the Rotterdam Study, a prospective

population-based cohort study that aims to assess the occurrence and risk factors of chronic diseases in the elderly.[13] The Rotterdam Study started in 1990 among 7,983 inhabitants of 55 years and older residing in Ommoord, a district of Rotterdam, the Netherlands. In 2000, the original cohort was extended with 3,011 participants who reached the age of 55 years or who had moved to the research area. In 2006, a third cohort started comprising 3,932 participants of 45 years and older. The studies included in this thesis are based on the original and first extension cohorts of the Rotterdam Study, which started in 1990 and 2000, respectively.

Chapter 2 focuses on the impact of established risk factors of dementia. In **Chapter 2.1**, I provide an overview of the current knowledge on the role of cardiovascular diseases and risk factors in the etiology of Alzheimer disease. I not only discuss clinical factors, but also markers of subclinical vascular disease, such as large vessel disease and cerebral small vessel disease. Many known risk factors of dementia are modifiable and in **Chapter 2.2**, I have calculated the proportion of dementia cases that could potentially be prevented if these factors would be entirely eliminated. As it is important to investigate early phases of the dementia syndrome, in **Chapter 2.3**, I investigate determinants, magnetic resonance imaging correlates, and the prognosis of MCI.

In **Chapter 3**, I then explore several emerging cardiovascular and metabolic factors. The relation between atrial fibrillation and dementia is described in **Chapter 3.1**, whilst the association between subclinical cardiac dysfunction and stroke and dementia is addressed in **Chapter 3.2**. In **Chapter 3.3**, I have investigated whether people with atherosclerotic calcification have an increased risk of dementia and cognitive decline. In **Chapter 3.4**, I describe the relation between cerebral vasomotor reactivity and mortality. **Chapter 3.5** focuses on the relation between insulin-like growth factor-I receptor stimulating activity and dementia.

In **Chapter 4**, I discuss the effect of several behavioral and emotional factors on the risk of dementia. I have studied the effect of physical activity on the risk of dementia in **Chapter 4.1**, the effect of anxiety on dementia in **Chapter 4.2**, and that of depression in **Chapter 4.3**.

Finally, in **Chapter 5**, I summarize the main findings of this thesis, discuss methodological issues concerning the studies described in this thesis and give potential implications for clinical practice and suggestions for further research.

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Chapter 2

The impact of conventional risk factors

Chapter 2.1

Cardiovascular risk factors and future risk of Alzheimer disease

Renée F.A.G. de Bruijn, M. Arfan Ikram

BMC Medicine 2014; 12:130

Abstract

Alzheimer disease (AD) is the most common neurodegenerative disorder in the elderly, but there are still no curative options. Senile plaques and neurofibrillary tangles are considered hallmarks of AD, but cerebrovascular pathology is also common in AD. In this review, we summarize findings on cardiovascular disease and risk factors in the etiology of AD. Firstly, we discuss the association of clinical cardiovascular diseases, such as stroke and heart diseases, and AD. Secondly, we summarize the relation between imaging makers of pre-clinical vascular disease and AD. Lastly, we discuss the association of cardiovascular risk factors and AD. We discuss both established cardiovascular risk factors and emerging putative risk factors, which exert their effect partly via cardiovascular disease.

Introduction

Alzheimer disease (AD) is the most common subtype of dementia and has a large patient and societal burden. AD has a complex and multifactorial etiology that involves senile plaques and neurofibrillary tangles.[1] Increasingly, the role of cardiovascular disease is also being recognized as an important etiologic hallmark of AD. Indeed, many studies have shown the importance of vascular pathology in AD.[2-7] As cardiovascular diseases have established therapeutic options and risk factors of cardiovascular disease are modifiable, focusing on the association between vascular pathology and AD might provide pathways to prevent or delay AD in the elderly.[8,9] In this narrative review, we provide an overview of the current knowledge on the relation between AD and clinical cardiovascular diseases, imaging markers of pre-clinical cardiovascular disease, and established and emerging cardiovascular risk factors (Table 1).

Table 1. List of potential vascular factors implicated in Alzheimer disease

Cardiovascular disease	Pre-clinical markers of cardiovascular disease	Established cardiovascular risk factors	Emerging risk factors
Stroke	Intima media thickness	Blood pressure, hypertension, and arterial stiffness	Inflammation
Atrial fibrillation	Carotid plaques	Glucose metabolism and diabetes mellitus	Chronic kidney disease
Coronary heart disease	Atherosclerotic calcification	Hypercholesterolemia	Thyroid function
Heart failure	Lacunes and white matter lesions	Smoking	
	Cerebral microbleeds	Obesity	
	Cerebral microinfarcts	Non-adherence to the Mediterranean diet and low levels of physical activity	
	Retinal vascular changes	Homocysteine	
	Microstructural integrity and connectivity		

Cardiovascular disease

Cardiovascular diseases, such as stroke, atrial fibrillation, coronary heart disease, and heart failure are very common in the elderly and have regularly been linked to AD. This association might be due to shared risk factors between cardiovascular diseases and AD, but there might also be a direct causal association since cardiac disease causes hypoperfusion and microemboli, which have been implicated in the etiology of AD.[10,11] In the following sections, we will discuss current evidence relating common cardiovascular diseases with risk of AD.

Stroke

Clinical stroke has often been associated with an increased risk of subsequent dementia, but this is by definition then termed post-stroke dementia or vascular dementia.[12] Such terminol-

ogy hampers thorough investigation of the role of clinical stroke in AD. Therefore, important evidence implicating stroke in the etiology of AD comes from studies investigating asymptomatic or silent stroke, which are often lacunes. Numerous studies have shown that lacunes strongly increase the risk of dementia, including AD.[13-15] Moreover, white matter lesions, which also represent ischemic brain damage, are also associated with cognitive impairment and AD.[16,17] These findings suggest that stroke is causally involved in the etiology of dementia. Mechanisms underlying this association include the following. Firstly, stroke causes loss of neuronal tissue which might enhance the degenerative effect of neuronal tissue loss due to amyloid and tau pathology.[15] Secondly, it has been suggested that cerebrovascular disease directly influences amyloid pathology due to accelerating amyloid β production or hampering amyloid β clearance[3,18], although studies on these pathways remain inconsistent.[3,18-21]

Atrial fibrillation

Several studies have shown that people with atrial fibrillation (AF) more often have AD and are at an increased risk of AD.[22-24] Because AF causes embolisms which could lead to stroke, the relation between AF and AD might be explained through clinical or silent stroke.[10,25-27] Accordingly, a meta-analysis showed that a consistent relation between AF and a higher risk of dementia was restricted to people with stroke.[23] However, another study found that stroke-free people with AF performed worse on memory and learning tasks, and had a reduced hippocampal volume.[28] Both memory function and hippocampal volume are strongly related to AD, which suggests there might be additional pathways explaining the association between AF and AD.[29] One hypothesis is that cerebral hypoperfusion in AF causes damage to nerve cells and thereby contributes to the etiology of AD.[23,25-27] Another hypothesis is that AF directly influences AD neuropathology, such as senile plaques and neurofibrillary tangles, but evidence for this explanation remains scarce.[30]

Coronary heart disease

Coronary heart disease (CHD) is the most common type of heart disease and one of the major causes of death worldwide.[31] CHD includes angina pectoris, myocardial infarction, and coronary revascularization procedures. The relation between CHD and AD remains difficult to disentangle because of strong competing risks of death: several studies showed that CHD is related to cognitive impairment or AD[32,33], whereas others found no association.[34,35] The Rotterdam Study showed that unrecognized myocardial infarction was associated with the risk of AD, whereas recognized myocardial infarction was not.[36] Explanations linking CHD with AD include shared etiology, as atherosclerosis plays an important role in both CHD and AD.[26,27] This hypothesis is corroborated by findings from the Cardiovascular Health Study, which showed that peripheral artery disease, another manifestation of atherosclerosis, was

also strongly associated with an increased risk of AD.[32] Furthermore, CHD might relate to AD through diminished cardiac function, hypoperfusion, and emboli.[10,25-27]

Heart failure

Heart failure represents a condition in which the pumping function of the heart is diminished and unable to supply the body with sufficient blood flow. Heart failure has been associated with cognitive impairment and AD.[37-39] A Swedish study found that heart failure was related to an increased risk of dementia, including AD.[37] The same study also found that treatment with antihypertensive drugs slightly reduced this risk. The Framingham Offspring study showed that even in people without clinical heart failure, lower cardiac function was related to lower brain volume, an important hallmark for dementia.[40] The pathways explaining the role of heart failure in the etiology of AD are similar to those of AF; heart failure results in hypoperfusion of the brain, which leads to hypoxia and damage to nerve cells.[3,4,25-27] Additionally, heart failure increases the risk of emboli and microvascular pathology, such as white matter lesions and lacunes, which in turn are related to an increased risk of dementia.[10,25-27]

Pre-clinical markers of cardiovascular disease

Cardiovascular pathology gradually accumulates over years before manifesting as a clinical event. Similarly, AD pathology also accumulates over decades before clinical symptoms occur. Consequently, several studies have sought to investigate how such pre-clinical pathology relates to cognitive decline and AD.

Pre-clinical markers of large vessel disease

Using various imaging techniques it is possible to assess markers of pre-clinical large vessel disease. Intima media thickness (IMT) and carotid plaque are measures of atherosclerosis in the carotid artery which can be obtained via ultrasonography. Both IMT and carotid plaque are more prevalent in patients with dementia and AD than in cognitively healthy people.[41] Moreover, both measures are related to increased cognitive decline in patients with AD.[42] Additionally, several population-based studies have shown that people with the highest IMT measures have an increased risk of incident dementia, including AD.[32,43,44] Carotid plaque scores were also associated with an increased risk of AD in one study, but this association lacked statistical significance.[44] Another marker of pre-clinical large vessel disease is calcification volume in the atherosclerotic plaque which can be assessed using Computed Tomography (CT). Although calcification is only part of the plaque, it is a suitable measure of the underlying plaque burden.[45] CT has the disadvantage of radiation exposure, but CT measures of atherosclerotic

calcification are more observer-independent than ultrasonography measures. Few studies have investigated the relation between CT-derived atherosclerotic calcification and dementia, but some studies found that larger calcification volumes in the coronary arteries, aortic arch, and carotid arteries relate to worse cognitive performance.[46,47] Moreover, larger calcification volume was associated with smaller brain tissue volumes and worse microstructural integrity of the white matter, which are both factors related to an increased risk of AD.[46] Mechanisms linking carotid large vessel disease to AD include sub-clinical cerebral small vessel disease (see below), hypoperfusion, or shared etiology.[3,4,6]

Pre-clinical markers of cerebral small vessel disease

Abundant evidence shows that structural imaging markers of cerebral small vessel disease, such as lacunes and white matter lesions, are related to cognitive impairment or AD.[15-17,48-50] Additionally, brain atrophy, which is an established marker of dementia and AD, is partly influenced by cardiovascular disease.[48,51,52] Cerebral microbleeds (CMBs) are an emerging vascular marker with great promise for AD research. Both amyloid β and vascular pathology are related to the etiology of CMBs, and therefore a link between CMBs and incident AD seems plausible.[53-55] However, this association still needs to be confirmed in longitudinal studies. In recent years it has also become possible to visualize cerebral microinfarcts using high-field magnetic resonance imaging (MRI) scanners, such as 7T scanners. The role of these microinfarcts in AD remains unclear, but is expected to be the focus of research in coming years.[56,57] Although it is possible to measure markers of cerebral small vessel disease, direct visualization of the small cerebral arterioles *in vivo* remains difficult. Retinal imaging provides an easy tool to visualize retinal vessels that originate embryologically from the same tissues as cerebral vessels. Thus, retinal imaging provides a possibility to study the small vessels of the brain *in vivo*. Retinal vessel diameter has been associated with white matter lesions, infarcts, brain atrophy, and an increased risk of vascular dementia.[58-60] Although a recent case-control study also found a link between AD and retinal microvascular changes[61], there is currently no evidence relating retinal vessels to an increased risk of AD longitudinally.

Measures of brain connectivity

In recent years, development of newer imaging techniques has allowed quantification of more subtle brain pathology such as changes in brain connectivity. Diffusion Tensor Imaging (DTI) assesses the microstructural integrity of the white matter and studies have suggested that DTI markers reflect a very early stage of vascular brain pathology. Consequently, several studies have shown loss of microstructural integrity in early AD or even in mild cognitive impairment (MCI).[62-64] However, longitudinal studies relating DTI-markers with incident AD are still largely lacking. Another novel MRI technique is resting-state functional MRI, which

measures brain function by functional connectivity at rest. Several studies have shown that functional connectivity is altered in patients with MCI and AD[65-69], but again robust longitudinal data are still lacking. Moreover, the role of cardiovascular risk factors in functional MRI remains unclear.

Cardiovascular risk factors

In addition to clinical cardiovascular diseases (see above), risk factors of cardiovascular disease have also been implicated in AD. The causal pathway of these risk factors might be associated with clinical diseases, but there is also evidence directly linking cardiovascular risk factors with AD.

Blood pressure, hypertension, and arterial stiffness

Several studies have related hypertension to brain atrophy, white matter lesions, and neurofibrillary tangles.[70-72] Therefore, an association between hypertension and AD is conceivable. Nonetheless, this association is complex and differs with age.[73] Several studies show midlife hypertension to be related to an increased risk of AD[74-77], whereas other studies failed to find an association between late-life hypertension and dementia. In fact, some studies even suggest low blood pressure might be related to AD.[73] These inconsistencies have yet not been elucidated, but it is suggested that blood pressure decreases in the years before clinical onset of dementia because of reduced physical activity and lowered body weight. Further research is still necessary to verify this hypothesis.[27]

A measure closely related to blood pressure and hypertension is arterial stiffness, which can be measured as increased pulse pressure or elevated pulse wave velocity. The difficulty in investigating arterial stiffness lies in the fact that it can be caused by hypertension as well as leading to hypertension.[78,79] Arterial stiffness results in an increased pulsatile pressure causing damage to the microvascular system of the brain[80], which in turn causes cognitive decline.[80] Indeed, some studies found a relation between higher pulse pressure or higher pulse wave velocity and an increased prevalence and risk of cognitive decline or AD[81-83]; however others could not demonstrate such an association.[84,85]

Glucose metabolism and diabetes mellitus

Diabetes mellitus is a complex disorder, in which insulin resistance leads to higher circulating blood glucose levels, which in turn lead to microvascular damage in various organs. In the brain, diabetes mellitus has been associated with infarcts and atrophy.[86,87] Accordingly, many studies have confirmed that the risk of dementia and AD is higher in people with diabetes

mellitus.[88] Furthermore, the risk of AD is also increased in people with borderline diabetes mellitus, that is, pre-diabetes.[89] Besides microvascular damage, other potential mechanisms relating diabetes mellitus with AD are direct neurotoxicity due to increased glucose insulin levels. A higher circulating blood glucose level is toxic to nerve cells as it causes protein glycation and oxidative stress.[88] Insulin is involved in amyloid β clearance from the brain and higher levels of insulin could disrupt this metabolism, leading to increased amyloid β burden.[88]

Hypercholesterolemia

Given the role of cholesterol in the clearance of amyloid β, hypercholesterolemia has been suggested as a risk factor for AD. Support for this hypothesis comes from a recent imaging study showing higher cholesterol levels to be related to higher amyloid β levels.[90] Similarly, *apolipoprotein* ε4-carrier status, one of the most important genetic risk factors of AD, is related to increased cholesterol levels.[91] However, results of epidemiological studies on the association between hypercholesterolemia and AD have been inconsistent. Some studies found that hypercholesterolemia in midlife was associated with an increased risk of AD, whereas in late-life there was no association.[92] An explanation is that a high cholesterol level in midlife is a risk factor of AD, whereas lower cholesterol levels in late-life probably reflect pre-clinical disease as lifestyle and dietary habits change in people with subclinical dementia.

Smoking

Various longitudinal studies have established smoking as a risk factor of dementia and AD.[93] Both the Rotterdam Study and the Honolulu-Asia Aging Study found that the risk of dementia in smokers was higher than that in non-smokers.[94,95] Furthermore, the Honolulu-Asia Aging Study found that number of pack-years was related to amyloid burden in the brain in a dose-response manner.[95] Smoking contributes to atherosclerosis and has been related to cerebral small vessel disease.[49,96] Additionally, tobacco contains many neurotoxins, which might cause direct neuronal damage.[97] However, the exact mechanisms underlying the relation between smoking and dementia require further investigation.

Obesity

Similar to hypertension and increased cholesterol levels, the association between obesity and risk of dementia and AD changes with age.[98-100] Obesity in midlife is associated with an increased risk of dementia and AD, whereas in older age a higher body weight seems to have a protective effect.[100,101] People with subclinical dementia gradually lose body weight due to altered life style and lowered food intake, and thus low body weight might also be an early symptom of dementia.[98-100] In contrast, midlife obesity increases the risk of many chronic

diseases, including vascular diseases, and could be related to an increased risk of dementia and AD via those pathways.[101]

Mediterranean diet and physical activity

The Mediterranean diet is characterized by a high intake of vegetables, fruits, cereals, and unsaturated fatty acids, a moderate intake of fish, poultry, eggs, red wine, and dairy products, and a low intake of saturated fats and red, processed meats.[102] Adherence to a Mediterranean diet has shown to reduce vascular disease and vascular risk factors, and to lower inflammation and oxidative stress.[103] Two recent meta-analyses concluded that adherence to a Mediterranean diet might reduce the risk of AD.[104,105] However, the number of studies with long follow-up is limited, and further research is necessary to confirm the potential protective effect of the Mediterranean diet on AD.

Besides dietary habits, another potential modifiable factor to reduce AD risk is physical activity.[9,106] Physical activity is inversely associated with cardiovascular disease and diabetes and could therefore also reduce the risk of AD.[107,108] Alternatively, physical activity could have a direct protective effect on the risk of dementia, as it improves cerebral perfusion and increases neurogenesis.[109,110] Several epidemiological studies have associated a higher level of physical activity with a reduced risk of dementia or cognitive decline.[111-115] However, most of these studies had relatively short follow-up, and studies with long follow-up periods have yielded inconsistent results.[115,116] For both physical activity levels and the Mediterranean diet, the possibility of reverse causality explaining short-term associations needs to be considered.[117]

Homocysteine

Plasma homocysteine levels reflect folate and vitamin B12 status, and are related to renal function. Increased homocysteine levels are associated with vascular disease and might have an effect on amyloid β and tau phosphorylation. Consequently, high plasma homocysteine levels have been related to an increased risk of AD.[118] Imaging and autopsy studies showed that increased homocysteine levels were associated with brain atrophy and neurofibrillary tangles.[119,120] However, not all studies concur with these results. A recent study found that plasma homocysteine levels were not related to AD, after adjusting for folate or vitamin B12 deficiency and renal dysfunction.[121] Further studies are needed to unravel this association.

Emerging risk factors

In addition to the classical vascular risk factors, there are other emerging risk factors that have been implicated in AD, partly via vascular mechanisms.

Inflammation

Various inflammatory markers have been related to an increased risk of dementia, including AD.[122-124] Astrocytes and microglia activate the neuronal immune system in response to pathogens such as infection and vascular pathology.[125,126] Several studies showed that senile plaques in the brains of patients with AD and of AD transgenic mice models were surrounded by an increased number of activated microglia.[127] Amyloid β also activates the neuronal immune system and might cause a chronic inflammatory reaction that has a toxic effect on nerve cells.[126] Moreover, recent genetic studies have uncovered various genes for inflammation and immune response that seem to be associated with AD.[128] However, there have been no major population-based cohort studies studying inflammation in AD, and trials studying the effect of immunotherapy on AD have not yet been successful.[126] Hence, further studies are required to elucidate the exact role of inflammation in AD.

Chronic kidney disease

In recent years, various studies have focused on the association between chronic kidney disease and cognitive decline or AD. Most[129-133], but not all[134], of these studies found that low kidney function was related to an increased risk of dementia, AD, or cognitive decline. These inconsistencies might be due to methodological discrepancies: different measures of kidney function were used and there was a large variation across study populations examined.[132] Mechanisms linking chronic kidney disease and dementia include shared risk factors (hypertension, arterial stiffness, smoking, and obesity) and direct consequences of chronic kidney disease (chronic inflammation, hemodynamic changes, anemia, and uremic toxins).[129] However, these pathways are not well established, and should be investigated further.

Thyroid function

Thyroid hormone is important for brain function, and thyroid dysfunction is a potentially reversible cause of cognitive impairment.[135] Thyroid hormone is involved in amyloid precursor protein (APP) regulation. Animal studies have shown that APP expression is increased in hypothyroidism, which leads to higher amyloid β levels.[135] In addition, thyroid dysfunction is associated with cardiovascular disease and could therefore influence AD pathology indirectly.[135] Lastly, thyroid hormone levels alter as a consequence of AD pathology through reduction in thyrotropin releasing hormone secretion.[136] Observational studies have shown both hypothyroidism and hyperthyroidism to be related to AD, but not all studies could establish an association.[136-140]

Conclusion

In conclusion, there is abundant and converging evidence showing that cardiovascular diseases and risk factors play an important role in the etiology of AD. While for some of these factors the mechanisms linking to AD are clear, for others the association with AD is more complex and needs further research to be completely unraveled. Nevertheless, given that these vascular factors are currently the only known modifiable risk factors for AD, the possibility of intervening with these factors to prevent or delay AD merits more dedicated research.

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Chapter 2.2

The potential for prevention of dementia across two decades

Renée F.A.G. de Bruijn, Michiel J. Bos, Marileen L.P. Portegies, Albert Hofman, Oscar H. Franco, Peter J. Koudstaal, M. Arfan Ikram

Submitted

Abstract

Background: Cardiovascular diseases, cardiovascular risk factors, and low education are important risk factors of dementia. We provide contemporary estimates of the proportion of dementia cases that could be prevented if potentially modifiable risk factors were eliminated, i.e. population attributable risk (PAR). Furthermore, we studied whether the PAR has changed across the last two decades.

Methods: This study is part of the Rotterdam Study. We included 7,003 participants of the original cohort and 2,953 participants of the extended cohort. The original cohort started in 1990 and the extended cohort in 2000. Both cohorts were followed for dementia until ten years after baseline. We calculated the PAR of overweight, hypertension, diabetes mellitus, cholesterol, smoking, and education. Additionally, we assessed the PAR of stroke, coronary heart disease, heart failure, and atrial fibrillation. We calculated the PAR for each risk factor separately and the combined PAR taking into account the interaction of risk factors.

Results: During 57,996 person-years of follow-up, 624 participants of the original cohort developed dementia, and during 26,177 person-years of follow-up, 145 participants of the extended cohort developed dementia. The combined PAR in the original cohort was 0.23 (95% CI 0.05;0.62). The PAR in the extended cohort was slightly higher at 0.30 (95% CI 0.06;0.76). The combined PAR including cardiovascular diseases was 0.25 (95% CI 0.07;0.62) in the original cohort and 0.33 (95% CI 0.07;0.77) in the extended cohort. An important limitation of our study is that we did not have information on all known modifiable risk factors which might have led to an underestimation of our results.

Conclusions: About one quarter to one third of dementia cases could be prevented if modifiable risk factors would be eliminated. Although prevention and treatment options of cardiovascular risk factors and diseases have improved, the preventive potential for dementia has not declined over the last two decades.

Introduction

Dementia is one of the most devastating diseases in the elderly. Although the etiology is still largely unknown, in recent years it has become clear that cardiovascular risk factors, cardiovascular diseases, and low educational level are risk factors of dementia.[1-3] Since most of these factors are potentially modifiable, there might be an opportunity to prevent dementia by timely targeting these risk factors. The recent World Alzheimer report is dedicated to this opportunity and advocates that there is convincing evidence that the risk of dementia can be modified via improved treatment of cardiovascular risk factors.[3] The magnitude of this potential for prevention can be estimated via the population attributable risk (PAR), which is the proportion of dementia cases that could be prevented by entirely eliminating modifiable risk factors and which depends on the relative risk and prevalence of these risk factors.[4]

Few studies have estimated PAR for modifiable risk factors of dementia.[5-10] However, results have been inconsistent due to differences in studied risk factors or the use of suboptimal statistical approaches. For instance, some studies estimated PAR using data from multiple different sources, which precludes proper adjustment for risk factors and only yields indirect approximations of PAR.[6,8,10] Nevertheless, the combined PAR of potentially modifiable risk factors of dementia has been estimated to range from 8.4 to 50.7%.[6-9]

At the same time, converging evidence, including form our own study, suggests that the incidence of dementia has declined over the last few decades.[11-15] Presumably, this decline has been primarily triggered by a better control of cardiovascular risk and improved education.[11-15] If this is indeed the case, it is conceivable that the previously published PARs of modifiable risk factors for dementia have now actually become an overestimation and that the proportion of dementia cases that can be additionally prevented by fully eliminating those risk factors is much lower. There is thus an urgent need for contemporary data on PAR of modifiable risk factors of dementia, because such updated knowledge can better inform public health priorities on preventive strategies against dementia.

In this study, we provide direct and contemporary estimates of PAR of modifiable risk factors of dementia. Additionally, we investigate how this PAR has changed across the last two decades.

Materials and Methods

Setting and study population

This study was conducted within the Rotterdam Study, a prospective population-based cohort study that aims to assess the occurrence and risk factors of chronic diseases in the elderly. The Rotterdam Study started in 1990 among inhabitants of 55 years and older residing in

Ommoord, a district of Rotterdam, the Netherlands. Of the 10,215 invited inhabitants, 7,983 agreed to participate in the baseline examinations. In 2000, the original cohort was extended with subjects who reached the age of 55 years and subjects who had moved to the research area. Of the 4,472 invitees, 3,011 agreed to participate. Follow-up examinations take place every three to four years. Details regarding the objective and design of the Rotterdam Study are described elsewhere.[16]

In the original cohort, we excluded 455 participants because they were not properly screened for dementia, 482 participants because they had prevalent dementia, and 43 participants for lack of follow-up information on the dementia diagnosis. Finally, 7,003 participants were included in the analyses. In the extended cohort, we excluded 29 participants because they had prevalent dementia and 29 for lack of follow-up information on the dementia diagnosis. Finally, 2,953 participants were included in the analysis.

The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.

Selection and measurement of risk factors

Potentially modifiable risk factors for dementia were chosen on the basis of previous literature.[1-3] The following risk factors were selected: overweight and obesity, hypertension, diabetes mellitus, unfavorable cholesterol levels, smoking, and low educational level.

All risk factors were measured at baseline. Weight and height were measured at the research center visit and body mass index (BMI) was calculated as weight in kilograms divided by length in meters squared. BMI was categorized into four categories: underweight (BMI <18.5), normal weight (BMI 18.5-25), overweight (BMI 25-30), and obesity (BMI >30). Blood pressure was measured in sitting position on the right arm and calculated as the average of two measurements using a random-zero sphygmomanometer. Hypertension was defined as a blood pressure ≥140/90 mmHg or use of blood pressure lowering medication, prescribed for the indication of hypertension. Diabetes mellitus was defined as a fasting serum glucose level ≥7.0 mmol/L, non-fasting serum glucose level ≥11.1 mmol/L, or use of anti-diabetic medication. Serum glucose, total cholesterol, and high-density lipoprotein (HDL)-cholesterol levels were acquired by an automated enzymatic procedure (Boehringer Mannheim System). Medication use, educational level, and smoking habits were assessed by interview. For people not taking lipid lowering medication, total cholesterol/HDL-cholesterol ratio was divided in quartiles using the lowest category as reference category. People using lipid lowering medication were added as fifth category and also compared to the reference category. Educational level was categorized as low (primary education or lower vocational education), intermediate (secondary education or intermediate vocational education), and high educational

level (higher vocational education or university). Smoking habits were categorized as current, former, and never smoking.
Although stroke, coronary heart disease, heart failure, and atrial fibrillation are cardiovascular diseases that may result from cardiovascular risk factors and can be modified only via secondary prevention, several studies have shown that these diseases are related to dementia or cognitive impairment, independently of cardiovascular risk factors.[17-20] We therefore assessed the PAR of these factors in an additional analysis. History of stroke, coronary heart disease (myocardial infarction or revascularization procedure), heart failure, and atrial fibrillation was evaluated using home interviews and confirmed by reviewing medical records.[21,22]

Assessment of dementia

Participants were screened for dementia at baseline and at follow-up examinations using a three-step protocol.[11] Screening was done using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Screen-positives (MMSE <26 or GMS organic level >0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[11] Participants who were suspected of having dementia underwent extra neuropsychological testing if necessary. Additionally, for persons not visiting the research center, the total cohort was continuously monitored for dementia through computerized linkage of the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. When information on neuroimaging was required and available, it was used for decision making on the diagnosis. In the end, a consensus panel, led by a neurologist, decided on the final diagnosis in accordance with standard criteria for dementia (DSM-III-R).[11] Both the original and the extended cohort were followed for dementia until ten years after baseline examinations: until 2000-2003 for the original cohort and until 2010-2012 for the extended cohort. Participants were censored within this follow-up period at date of death, date of loss to follow-up, or the end of the study period, whichever date came first. Follow-up for dementia was complete for 99.5% of potential person-years in the original cohort and for 98.5% of potential person-years in the extended cohort.

Statistical analyses

We imputed missing data on the investigated risk factors (3.3% in the original cohort and 6.7% in the extended cohort) using the mean of five imputations. Missing data were imputed on age, sex, and all other investigated risk factors. Differences between the original and the extended cohort were calculated using logistic regression models, adjusting for age and sex where appropriate. Analyses were performed using statistical software package SPSS 20.0.
We calculated PARs and corresponding 95% confidence intervals (CIs) using the Interactive

Risk Attributable Program (US National Cancer Institute).[23] This metric is also referred to as population attributable fraction, or PAR%. We provide logit transformed 95% CIs accompanying the PARs as these are better interpretable and more stringent with regard our combined PAR estimates.[24] The PAR was estimated and adjusted for confounding according to the following formulas:

$$\mathrm{PAR} = 1 - \sum_{i=1}^{I} \sum_{j=1}^{J} \rho_{ij} R_{i|j}^{-1} \tag{1}$$

where

$$R_{i|j} = \frac{\Pr(D=1 \mid X=x_i, C=c_j)}{\Pr(D=1 \mid X=x_1, C=c_j)} \tag{2}$$

and

$$\rho_{ij} = \Pr(X=x_i, C=c_j \mid D=1) \tag{3}$$

with D=1 denoting presence of disease, X denoting exposure with i levels, and C denoting a confounder with j levels. The relative risk was estimated from a multivariable Poisson model.[23] First, we calculated the PAR for overweight and obesity, hypertension, diabetes mellitus, unfavorable cholesterol levels, smoking, and low educational level. Second, we calculated the PAR of stroke, coronary heart disease, heart failure, and atrial fibrillation. We calculated the PAR for each risk factor separately as well as the combined PAR per cohort. Many risk factors have a coinciding effect on the etiology of dementia. Therefore, the combined PAR cannot be estimated by the sum of the separate PARs as this would lead to an overestimation. The advantage of the statistical program used in this study is that it takes into account the overlap between the PARs of each risk factor when estimating the combined PAR. The combined PAR included the PARs of modifiable risk factors which were associated with dementia in the expected direction in our study, meaning the relative risk was above one. PARs cannot be calculated using a relative risk below one as this will result in a PAR which cannot be interpreted.[25]

Every PAR was adjusted for age, sex, and all other risk factors included in the model. Age was added per five year categories into the models. Because of a small number of people in the oldest age categories, the oldest age category of both cohorts comprised people 85 years of age and older.

Results

Baseline characteristics

Baseline characteristics of the two study populations are provided in Table 1. During a mean follow-up of 8.3 years (standard deviation (SD) 2.9), 624 participants of the original cohort developed dementia. During a mean follow-up of 8.9 years (SD 2.3), 145 participants of the extended cohort developed dementia. Cut-offs for quartiles of total cholesterol/HDL-cholesterol ratio were: 4.1, 5.0, and 6.1 in the original cohort and 3.6, 4.3, and 5.2 in the extended

Table 1. Baseline characteristics of the original and the extended cohort of the Rotterdam Study

	RS-I N=7,003	RS-II N=2,953	P-value for difference
Age, years	69.4 (9.1)	65.0 (8.3)	<0.001
Sex, female	4,187 (59.8%)	1,661 (56.2%)	0.15
Body mass index			
<18.5	69 (1.0%)	15 (0.6%)	0.65
18.5-25	2,486 (37.5%)	790 (29.9%)	Reference
25-30	3,097 (46.7%)	1,286 (48.6%)	<0.001
>30	977 (14.7%)	555 (21.0%)	<0.001
Hypertension	3,793 (56.3%)	1,688 (61.9%)	<0.001
Diabetes mellitus	727 (10.5%)	319 (10.9%)	0.001
Total cholesterol, mmol/L	6.6 (1.2)	5.8 (1.0)	<0.001
HDL-cholesterol, mmol/L	1.3 (0.4)	1.4 (0.4)	0.004
Lipid lowering medication	164 (2.3%)	367 (12.5%)	<0.001
Smoking			
Former	2,848 (41.7%)	1,381 (47.4%)	0.001
Current	1,560 (22.8%)	678 (23.3%)	0.60
Educational level			
Intermediate	2,540 (37.3%)	1,429 (49.4%)	<0.001
Low	3,700 (54.3%)	975 (33.7%)	<0.001
Stroke	175 (2.5%)	94 (3.2%)	<0.001
Coronary heart disease	535 (8.4%)	163 (6.1%)	0.04
Heart failure	220 (3.3%)	31 (1.2%)	0.01
Atrial fibrillation	316 (4.9%)	79 (3.6%)	0.44

Data are presented as means (standard deviations) or numbers (percentages). Percentages are calculated without missing data.

Abbreviations: RS-I=Rotterdam Study I, original cohort; RS-II=Rotterdam Study II, extended cohort; N=number of participants; HDL=high-density lipoprotein. Differences between the original and extended cohort were calculated using logistic regression models, adjusting for age and sex where appropriate.

cohort. Participants in the extended cohort were younger and had higher educational levels than those in the original cohort. Overweight and obesity, hypertension, diabetes mellitus, use of lipid lowering medication, former smoking, and stroke were more prevalent in the extended cohort. Additionally, people had lower total cholesterol levels, higher HDL-cholesterol levels, and less heart failure and coronary heart disease in the extended cohort than in the original cohort (Table 1).

Population attributable risk

In the original cohort, smoking (PAR 0.07, 95% CI 0.02;0.23) and lower educational level (PAR 0.07, 95% CI 0.00;0.90) had the largest PARs, followed by diabetes mellitus (PAR 0.04, 95% CI 0.01;0.09), hypertension (PAR 0.04, 95% CI 0.00;0.44) and higher total cholesterol/HDL-cholesterol ratio (PAR 0.03, 95% CI 0.00;0.73) (Table 2). Overweight and obesity were not related to dementia in the expected direction. The combined PAR in the original cohort was 0.23 (95% CI 0.05;0.62) (Table 2).
In the extended cohort, hypertension (PAR 0.16, 95% CI 0.02;0.62), lower educational level (PAR 0.12, 95% CI 0.00;0.89), and diabetes mellitus (PAR 0.06, 95% CI 0.02;0.19) had the largest effect on the burden of dementia (Table 2). Overweight and obesity, higher total cholesterol/HDL-cholesterol ratio, and smoking were not related to dementia in the expected direction. The combined PAR in the extended cohort was 0.30 (95% CI 0.06;0.76) (Table 2).
When we included cardiovascular diseases into our model, the combined PAR was 0.25 (95% CI 0.07;0.62) in the original cohort and 0.33 (95% CI 0.07;0.77) in the extended cohort. Atrial fibrillation and stroke had the largest PARs in the original cohort, whereas coronary heart disease and stroke had the largest PARs in the extended cohort (Table 3).

Discussion

Within the population-based Rotterdam Study, we found that about one quarter to one third of dementia cases could potentially be prevented through optimal prevention or treatment of cardiovascular risk factors and diseases and improvement of educational level. This proportion has not declined between the original cohort from 1990-2000 and the extended cohort from 2000-2010, although we did observe a shift in the relative importance of individual factors.
Before these results can be interpreted, some methodological issues need to be mentioned. Strengths of our study are the prospective, population-based design and almost complete dementia case finding. Additionally, information on a relatively wide range of risk factors was assessed, allowing us to calculate PARs on various potentially modifiable risk factors. Furthermore, we used a statistical approach which takes into account the interaction between risk factors when estimating the combined PAR.

Table 2. Population attributable risk of risk factors in the original and extended cohort of the Rotterdam Study

	RS-I n/N 624/7,003			RS-II n/N 145/2,953		
	HR (95%CI)	PAR (95%CI) per category	Combined PAR (95%CI)	HR (95%CI)	PAR (95%CI) per category	Combined PAR (95%CI)
Body mass index			NA			NA
<18.5	1.25 (0.62;2.56)	0.00 (0.00;0.08)		1.41 (0.19;10.49)	0.00 (0.00;0.67)	
25-30	0.96 (0.81;1.15)	NA		0.90 (0.61;1.32)	NA	
>30	0.84 (0.65;1.08)	NA		0.81 (0.48;1.36)	NA	
Hypertension	1.07 (0.89;1.27)		0.04 (0.00;0.44)	1.25 (0.81;1.94)		0.16 (0.02;0.62)
Diabetes mellitus	1.31 (1.05;1.63)		0.04 (0.01;0.09)	1.51 (0.96;2.37)		0.06 (0.02;0.19)
Cholesterol/HDL-cholesterol			0.03 (0.00;0.73)			NA
Quartile 2	1.04 (0.83;1.30)	0.01 (0.00;0.82)		0.64 (0.39;1.04)	NA	
Quartile 3	1.08 (0.86;1.35)	0.02 (0.00;0.30)		0.86 (0.53;1.39)	NA	
Quartile 4	1.04 (0.83;1.32)	0.01 (0.00;0.70)		0.72 (0.42;1.21)	NA	
Lipid lowering medication	0.64 (0.28;1.45)	NA		1.03 (0.60;1.74)	0.00 (0.00;1.00)	
Smoking			0.07 (0.02;0.23)			NA
Former	1.10 (0.90;1.34)	0.03 (0.00;0.23)		1.00 (0.68;1.48)	0.01 (0.00;1.00)	
Current	1.33 (1.04;1.71)	0.04 (0.02;0.10)		0.86 (0.48;1.52)	NA	
Educational level			0.07 (0.00;0.90)			0.12 (0.00;0.89)
Intermediate	0.99 (0.68;1.45)	NA		1.01 (0.57;1.78)	0.00 (0.00;1.00)	
Low	1.13 (0.78;1.63)	0.08 (0.00;0.66)		1.32 (0.73;2.36)	0.11 (0.02;0.51)	
Total			0.23 (0.05;0.62)			0.30 (0.06;0.76)

Estimates represent hazard ratios (95% confidence intervals) and population attributable risks (95% confidence intervals). Models were adjusted for age, sex, and for body mass index, hypertension, diabetes mellitus, total cholesterol/HDL-cholesterol ratio, lipid lowering medication, smoking, and educational level, if appropriate. For some risk factors, PARs could not be calculated as these risk factors were not related to dementia in the expected direction; these PARs are therefore not applicable (indicated with NA).[25]
Abbreviations: RS-I=Rotterdam Study I, original cohort; RS-II=Rotterdam Study II, extended cohort; HR=hazard ratio; CI=confidence interval; n=number of cases; N=number of people at risk; PAR=population attributable risk; NA=not applicable; HDL=high-density lipoprotein.

Table 3. Population attributable risk of cardiovascular diseases in the original and extended cohort of the Rotterdam Study

	RS-I n/N 624/7,003		RS-II n/N 145/2,953	
	HR (95%CI)	**Combined PAR (95%CI)**	**HR (95%CI)**	**Combined PAR (95%CI)**
Total without CVD		0.23 (0.05;0.62)		0.30 (0.06;0.76)
Stroke	1.43 (1.00;2.04)	0.02 (0.00;0.05)	1.70 (0.86;3.37)	0.03 (0.01;0.13)
Coronary heart disease	1.00 (0.73;1.38)	0.00 (0.00;1.00)	1.38 (0.79;2.43)	0.03 (0.00;0.20)
Heart failure	0.87 (0.59;1.28)	NA	0.33 (0.04;2.39)	NA
Atrial fibrillation	1.32 (0.99;1.77)	0.02 (0.01;0.06)	0.36 (0.13;0.97)	NA
Total including CVD		0.25 (0.07;0.62)		0.33 (0.07;0.77)

Estimates represent hazard ratios (95% confidence intervals) and population attributable risks (95% confidence intervals). Models were adjusted for age, sex, and for body mass index, hypertension, diabetes mellitus, total cholesterol/HDL-cholesterol ratio, lipid lowering medication, smoking, educational level, stroke, coronary heart disease, heart failure, and atrial fibrillation if appropriate. For some risk factors, PARs could not be calculated as these risk factors were not related to dementia in the expected direction; these PARs are therefore not applicable (indicated with NA).[25] Abbreviations: RS-I=Rotterdam Study I, original cohort; RS-II=Rotterdam Study II, extended cohort; n=number of cases; N=number of people at risk; HR=hazard ratio; CI=confidence interval; PAR=population attributable risk; CVD=cardiovascular disease; NA=not applicable.

We recognize that our study also has limitations. PAR calculations should always be interpreted with caution as they rely on assumptions which may be improbable in practice.[26,27] For instance, the model assumes that a risk factor can be successfully treated in every person, which is unlikely. Furthermore, PAR calculations assume that when a risk factor is successfully treated, the harming effect of the risk factor completely disappears, which too is unlikely as risk factors might have already caused irreversible damage before treatment is started. Therefore, the proportion that can actually be prevented in practice might be lower than suggested by the PAR.[26,27] We selected risk factors of dementia based on previous literature.[1-3] In our study, we did not find many statistically significant associations between these risk factors and the risk of dementia. This might have various explanations. First, dementia is a multifactorial disease and the effect of a single risk factor can therefore be relatively small and non-significant. However, the preventive potential of all risk factors combined can still be substantial. Second, our sample size was limited, which might have prevented us from finding statistically significant results. Third, it has been suggested that the effect of several cardiovascular risk factors on the risk of dementia changes with increasing age. Studies have shown that obesity, hypertension, and high cholesterol levels were only related to an increased risk of dementia when assessed at midlife.[28-30] Since the participants in our study populations were older, we were not able to assess such midlife effects, possibly leading to an underestimation of our results. Although our data collection was extensive, we did not have information on some

important modifiable risk factors, such as physical activity, dietary habits, depression, and social engagement, which might have led to an underestimation of our results. We imputed missing data on investigated risk factors, which may have introduced some misclassification. However, we hypothesized that excluding participants with missing data from our analyses would have resulted in more bias, given that missing data usually not occurs at random. A final limitation is that most participants of the Rotterdam Study are Caucasians and live in a middle income district of Rotterdam, which limits the generalizability of our results.

We found that the PAR of modifiable risk factors of dementia was about one quarter to one third and has not declined across the last two decades. This finding has several implications. First, from a public health point of view, this suggests that despite the seemingly declining incidence of dementia, the potential for further reduction of dementia is still substantial. Second, it remains pivotal to find novel risk factors that can explain the remaining two thirds of dementia cases. Third, even though the combined PAR did not decline, we did find that the contribution of individual risk factors had changed across the two decades. For example, the PARs of hypertension and to a lesser extent of diabetes were higher in the extended cohort. Although treatment and preventive options for cardiovascular risk factors have improved over the past decades, the prevalence of various cardiovascular risk factors, such as hypertension, diabetes, and obesity has increased.[31-33] Correspondingly, we also found that participants in the extended cohort had a higher prevalence of these risk factors, partly explaining the higher PARs. Conversely, we found a decline in the PARs of other cardiovascular risk factors, such as smoking and unfavorable cholesterol levels. Smoking had a large effect on the burden of dementia in the original cohort, which decreased dramatically in the extended cohort. Successful anti-smoking campaigns are an obvious explanation for these findings. A counterargument, however, is that the prevalence of current smokers was comparable between the two cohorts. The reduction of the effect of unfavorable cholesterol levels might be explained by the large increase in use of lipid lowering medication in the extended cohort. Another interesting observation was the effect of educational level, which was higher in the extended cohort than in the original cohort. Conventionally, educational attainment is considered a reflection of cognitive reserve built up earlier in life. However, this has not always been the case in older Dutch generations, where many people were unable to achieve their educational potential due to the Second World War. This might have led to a discrepancy between educational attainment and corresponding cognitive reserve. In the original cohort of the Rotterdam Study, this phenomenon might have been more pronounced, explaining the higher prevalence but lower PAR of low educational level compared to the extended cohort.

As for cardiovascular diseases, atrial fibrillation had an effect on the burden of dementia in the original cohort, but not in the extended cohort. These results might be explained by improved prevention of ischemic stroke in patients with atrial fibrillation.[34] In contrast, we found a stronger effect of stroke and coronary heart disease in the extended cohort than in the

original cohort. This might seem counter-intuitive, since preventive and treatment options for these diseases have improved in the same time-period. However, these findings could be explained by the fact that because of improved treatments, people with cardiovascular disease live longer and therefore are at an increased risk of developing dementia.[35]

The combined PAR we found is very much in line with a recent report that was based on meta-analyses of studies, which mostly used data from the nineties.[8] We calculated PARs directly using original data from 1990-2000 and 2000-2010 and more importantly took into account interaction between risk factors. Our study therefore adds important veracity to the estimates of PAR. However, given that PAR calculations rely on theoretical assumptions, future research is necessary to observe the actual effect of risk factor improvement on the risk of dementia. Furthermore, other studies should also focus on identifying novel modifiable risk factors for dementia.

In conclusion, we found that the potential of prevention of dementia through proper control of modifiable risk factors is about one quarter to one third and has not declined over the last two decades. As this is currently the only option to diminish the burden of dementia, public health interventions are urgently needed.

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Chapter 2.3

Determinants, MRI-correlates, and prognosis of mild cognitive impairment

Renée F.A.G. de Bruijn*, Saloua Akoudad*, Lotte G.M. Cremers*, Albert Hofman, Wiro J. Niessen, Aad van der Lugt, Peter. J. Koudstaal, Meike W. Vernooij, M. Arfan Ikram

*These authors contributed equally to the manuscript

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Abstract

Background: Mild cognitive impairment (MCI) marks a transitional stage between healthy aging and dementia, but the understanding of MCI in the general population remains limited. We investigated determinants, MRI-correlates, and prognosis of MCI within the population-based Rotterdam Study.

Methods: We studied age, *APOE*-ε4 carriership, waist circumference, hypertension, diabetes mellitus, total and HDL-cholesterol levels, smoking, and stroke as potential determinants of MCI. Determinants were assessed cross-sectionally at baseline (2002-2005) and up to 7 years prior to baseline (1997-2001). In addition, we compared volumetric, microstructural, and focal MRI-correlates in persons with and without MCI. Lastly, participants were followed for incident dementia and mortality until 2012 and we determined whether MCI was related to an increased risk of dementia and mortality.

Results: Out of 4,198 participants, 417 had MCI, of whom 163 amnestic and 254 non-amnestic MCI. At baseline, older age, *APOE*-ε4 carriership, lower total cholesterol levels, and stroke were associated with MCI. Additionally, lower HDL-cholesterol levels and smoking were related to MCI when assessed 7 years prior to baseline. Persons with MCI, particularly those with non-amnestic MCI, had larger white matter lesion volumes, worse microstructural integrity of normal-appearing white matter, and a higher prevalence of lacunes, compared to cognitively healthy participants. MCI was associated with an increased risk of dementia (HR 3.98, 95% CI 2.97;5.33), Alzheimer disease (HR 4.03, 95% CI 2.92;5.56), and mortality (HR 1.54, 95% CI 1.28;1.85).

Conclusions: Several vascular risk factors and MRI-correlates of cerebrovascular disease were related to MCI in the general population. Participants with MCI had an increased risk of dementia, including Alzheimer disease, and mortality.

Introduction

Although the etiology of dementia is largely unknown, it is well established that neuropathology related to dementia slowly accumulates over decades. Consequently, identifying persons at a higher risk of dementia could postpone or even prevent dementia by timely targeting modifiable risk factors.[1] In this light, mild cognitive impairment (MCI) has been identified as the transitional stage between normal aging and dementia.

Thus far, several studies have focused on identifying determinants, magnetic resonance imaging (MRI)-correlates, and prognosis of MCI. Various studies have established the role of amyloid pathology in MCI, but emerging evidence also implicates vascular factors as risk factors for MCI.[2-4] However, findings on determinants, MRI-correlates, and prognosis of MCI vary greatly due to differences in study populations, definitions of MCI, and determinants under investigation.[2,5-11] Studying MCI in the general population may strengthen previous findings on determinants and prognosis of MCI. More importantly, clinical studies may suffer from referral bias and reverse causality. In the general population referral bias is less present and investigating determinants years before MCI could overcome the problem of reverse causality.

In the population-based Rotterdam Study we investigated determinants, MRI-correlates, and prognosis of MCI. Firstly, we focused on several vascular risk factors that were measured not only cross-sectionally, but also up to 7 years prior to diagnosis of MCI. Secondly, we investigated the relation between MCI and volumetric, microstructural, and focal imaging markers. Thirdly, we followed participants over a period of 9 years to determine the risk of incident dementia, Alzheimer disease, and mortality.

Materials and methods

Setting and study population

The Rotterdam Study is a prospective population-based cohort that started in 1990. Inhabitants, aged 55 years and older, of Ommoord, a district of Rotterdam, the Netherlands were invited to participate in the study.[12] Out of 10,215 invited inhabitants, 7,983 (78%) agreed to participate. In 2000, this cohort was extended with 3,011 participants (67% of invitees) who had become 55 years of age or had moved into the district since the start of the study. Every 4 years, participants are re-invited to undergo home interviews and various examinations at the research center.[12]

Between 2002-2005, which was the fourth examination round of the original cohort and the second examination round of the extended cohort, an extensive neuropsychological test battery was implemented in the Rotterdam Study.[12] Given that extensive neuropsychological

testing is required to assess MCI, 2002-2005 was set as baseline for MCI screening in our study. Of the 6,061 study participants that underwent examinations between 2002-2005, 192 participants were excluded because they were demented, 67 because they were not sufficiently screened for dementia, and another 250 participants because they did not answer the questions regarding subjective cognitive complaints. An additional 1,354 participants were excluded because they missed one or more cognitive test scores or had unreliable test scores. Eventually, 4,198 people were eligible to participate in this study (Supplementary figure 1). Because MRI was implemented from 2005 onwards[13], only a random subset of 697 out of 4,198 people underwent MRI, which was on average 1.01 years (0.46 standard deviation (SD)) after MCI screening. People with cortical infarcts were excluded (N=15) as tissue loss and gliosis surrounding cortical infarcts may cause unreliable white matter lesion segmentations. Eventually, 682 participants were included in the analyses of MRI-correlates (Supplementary figure 1).

Determinants of MCI and other measurements

Determinants of MCI were selected based on biological plausibility and literature on established risk factors of dementia.[14-18] Educational level was assessed at study entry by interview and categorized into seven groups: primary education only or primary education with an unfinished higher education, lower vocational education, lower secondary education, intermediate vocational education, general secondary education, higher vocational education, and university. Since educational level was required for assessment of the MCI diagnosis, we imputed missing values for education (1.8%) based on age and sex.

Information on *APOE* genotype was obtained using polymerase chain reaction on coded DNA samples without knowledge of MCI diagnosis. This method has been described in detail previously.[19,20] *APOE*-ε4 carrier status was defined as carrier of one or two ε4 alleles. Waist circumference was measured in centimeters at the level midway between the lower rib margin and the iliac crest, with participants in standing position without heavy outer garments and with emptied pockets while breathing out gently. Blood pressure was measured in sitting position on the right arm and calculated as the average of two measurements using a random-zero sphygmomanometer. Hypertension was defined as a blood pressure ≥140/90 mmHg or use of blood pressure lowering medication, prescribed for the indication of hypertension. Diabetes mellitus was defined as a fasting serum glucose level ≥7.0 mmol/L, non-fasting serum glucose level ≥11.1 mmol/L, or use of anti-diabetic medication. Serum glucose, total cholesterol, and HDL-cholesterol levels were acquired by an automated enzymatic procedure (Boehringer Mannheim System). Smoking habits were assessed by interview and categorized as current, former, and never smoking. At study entry, history of stroke was assessed using home interviews and confirmed by reviewing medical records. After entering the Rotterdam Study,

participants were continuously followed-up for stroke through automatic linkage of general practitioner files with the study database. For potential strokes, additional information was collected from hospital, nursing home, and general practitioner records. An experienced neurologist adjudicated the strokes using standardized definitions, as described in detail previously.[21]
Apart from educational level and *APOE*-ε4 carrier status, all measurements were assessed at each examination round of the Rotterdam Study. We used the measurements that were assessed at the baseline of this study (2002-2005), and the measurements that were assessed at the previous examination round, which was up to 7 years (mean 4.36 years, SD 0.55) prior to baseline (1997-2001) (Supplementary figure 1).

Assessment of MCI

MCI was defined using the following criteria: 1) presence of subjective cognitive complaints, 2) presence of objective cognitive impairment, and 3) absence of dementia.
Subjective cognitive complaints were evaluated by interview. This interview included three questions on memory (difficulty remembering, forgetting what one had planned to do, and difficulty finding words), and three questions on everyday functioning (difficulty managing finances, problems using a telephone, and difficulty getting dressed). Subjective cognitive complaints were scored positive when a subject answered "yes" to at least one of these questions. We assessed objective cognitive impairment using a cognitive test battery comprising letter-digit substitution task, Stroop test, verbal fluency test, and 15-word verbal learning test based on Rey's recall of words.[22] To obtain more robust measures, we constructed compound scores for various cognitive domains including memory function, information-processing speed, and executive function.[22,23] Briefly, compound score for memory was calculated as the average of Z-scores for the immediate and delayed recall of the 15-word verbal learning test. For information processing speed averaged Z-scores for the Stroop reading and Stroop colour-naming subtask and the letter-digit substitution task were used. Finally, executive function included Z-scores of the Stroop interference subtask, the letter-digit substitution task, and the verbal fluency test. We classified people as cognitively impaired if they scored below 1.5 SD of the age and education adjusted means of the study population. We subsequently classified the MCI subtypes amnestic and non-amnestic MCI. Amnestic MCI was defined as people with MCI who had an impaired test score on memory function (irrespective of other domains). Non-amnestic MCI was defined as people with MCI having normal memory function, but an impaired test score on executive function or information-processing speed.

Brain MRI and post-processing

We performed a multisequence MRI protocol on a 1.5-Tesla scanner (GE Healthcare). The

sequences in the imaging protocol consisted of three high resolution axial scans, i.e., a T1-weighted sequence (slice thickness 1.6 mm, zero-padded to 0.8), a proton density-weighted sequence (slice thickness 1.6 mm), and a fluid-attenuated inversion recovery (FLAIR) sequence (slice thickness 2.5 mm).[13] For cerebral microbleed detection, we used a custom-made accelerated three-dimensional T2*-weighted gradient-recalled echo (3D T2* GRE (slice thickness 1.6 mm, zero-padded to 0.8).[24] For diffusion tensor imaging (DTI) scans we used a 2D acquisition and EPI readout (slice thickness for DTI was 3.5 mm). Maximum b-value was 1000 s/mm^2 in 25 non-collinear directions (number of excitations (NEX)=1) and one volume was acquired without diffusion weighting (b-value= 0 s/mm^2).

We used an automated tissue segmentation, including conventional k-nearest-neighbor brain tissue classifier extended with white matter lesion (WML) segmentation[25], to segment scans into grey matter volume, white matter volume, WML volume, cerebrospinal fluid, and background. Total brain volume was defined as the sum of total grey matter volume, white matter volume, and WML volume. Hippocampal volume was determined using an automated method, as described extensively before.[26]

The segmentation was brought to the DTI image space using boundary based registration performed on the white matter segmentation[27], the b=0 and T1-weighted images.

Diffusion data was pre-processed using a standardized processing pipeline.[28] In short, DTI data was corrected for subject motion and eddy currents by affine co-registration of the diffusion weighted volumes to the b=0 volume, including correction of gradient vector directions. Diffusion tensors were estimated using a non-linear Levenberg Marquadt estimator, available in ExploreDTI.[29] Global fractional anisotropy (FA) and mean diffusivity (MD), measures of microstructural integrity, were computed from the estimated tensor images over the entire normal-appearing white matter in each subject. The final registration result of each scan was checked visually for errors.[30,31] Partial volume effects and presence of multiple white matter fibre orientation within a voxel were thus minimized. Sixteen subjects had to be excluded from the white matter microstructural integrity analyses due to scanning artifacts or excessive motion. FA and MD were standardized to echo time (TE) values, because TE was not constant for all participants.

All scans were rated by 1 of 5 trained research-physicians to determine presence of microbleeds and lacunes of presumed vascular origin.[32] Microbleeds were rated as focal areas of signal loss, on 3D T2* Gradient Recalled Echo-weighted MRI. Lacunes were rated on FLAIR, proton-density-weighted and T1-weighted sequences, and were defined as focal lesions ≥3mm and <15mm in size, with the same signal intensity as cerebrospinal fluid on all sequences and a hyperintense rim on the FLAIR (when located supratentorially).[33] Infarcts showing involvement of grey matter were classified as cortical infarcts.

Assessment of dementia

Participants were screened for dementia at baseline and at follow-up examinations using a three-step protocol. Screening was done using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level.[34,35] Screen-positives (MMSE <26 or GMS organic level >0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[36] During this interview, more information on functional status and cognitive performance was collected. Participants who were suspected of having dementia underwent extra neuropsychological testing if necessary. Additionally, for people not visiting the research center, the total cohort was continuously monitored for dementia through computerized linkage of the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. When information on neuroimaging was required and available, it was used for decision making on the diagnosis. In the end, a consensus panel, led by a neurologist, decided on the final diagnosis in accordance with standard criteria for dementia (Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R)) and Alzheimer Disease (National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)).[37,38] Follow-up for incident dementia was complete until January 1st, 2012.

Assessment of mortality

Deaths were continuously reported through automatic linkage with general practitioner files. In addition, municipal health records were checked bimonthly for information on vital status. Information about cause and circumstances of death was obtained from general practitioner and hospital records.[39] Follow-up for mortality was complete until January 1st, 2012.

Statistical analysis

Firstly, we examined whether risk factors of dementia were related to MCI using multivariate logistic regression models adjusted for age and sex cross-sectionally. Since vascular risk factors often correlate[17], we estimated the independent effect of each risk factor by including all risk factors into the same model. Age was included per 5 year increase into the model and waist circumference, total and HDL-cholesterol levels were included per SD increase into the model. People with missing values were excluded from these analyses. We investigated whether excluded persons had different characteristics than people who were included in the analysis using Univariate Analysis of Variance, adjusting for age and sex where appropriate. The same models as in the cross-sectional analysis were used to examine the relation with risk factors assessed up to 7 years prior to MCI diagnosis. Secondly, we used linear and

logistic regression to investigate the relation of MCI with volumetric markers (i.e., total brain volume, hippocampal volume, WML volume), microstructural integrity markers (i.e., mean FA, MD) and focal markers (i.e, cerebral microbleeds, lacunes) of brain pathology on MRI cross-sectionally. Hippocampal volume was studied as the mean of the left and right hippocampal volume. WML was log-transformed due to the skewed distribution. Volumetric and microstructural measures were modeled continuously. Microbleeds and lacunes were dichotomized into present versus absent. These analyses were adjusted for age and sex (model I), and additionally for *APOE*-ε4 carriership, waist circumference, hypertension, diabetes mellitus, total and HDL-cholesterol levels, and smoking (model II). In model II we investigated whether irrespective of the presence of vascular risk factors, people with MCI had more volumetric, microstructural, and focal changes in the brain compared to cognitively healthy participants. Analyses of volumetric and microstructural integrity measures were also adjusted for intracranial volume. In addition we performed a sensitivity analysis for the imaging correlates, excluding participants who became demented in the period between MCI screening and MRI scanning (N=12). Thirdly, we used Cox proportional hazards to study the association between MCI and risk of dementia, Alzheimer disease, and mortality longitudinally. These models were adjusted for the same determinants as described in model II but with addition of prevalent stroke and educational level. All analyses were repeated investigating the amnestic and non-amnestic MCI subtypes separately. Analyses were performed using statistical software package SPSS 20.0, using an α-value of 0.05.

Results

Characteristics of the study population are presented in Table 1. Out of 4,198 participants, 417 (9.9%) had MCI. Of these, 163 had amnestic MCI and 254 had non-amnestic MCI. Missing values for determinants of MCI occurred in 268 participants (6.4%) in 2002-2005 (baseline) and in 615 participants (14.6%) in 1997-2001. Participants excluded from the baseline analyses were more often female, suffered more from hypertension, and had a larger waist circumference than participants included in the analyses. Participants excluded due to missing data in 1997-2001 were also more often female and more often hypertensive but had lower cholesterol levels than participants included in our analyses (Supplementary table 1).
At baseline, older age (odds ratio (OR) per 5 year increase in age 1.20, 95% confidence interval (CI) 1.11;1.29), *APOE*-ε4 carriership (OR 1.26, 95% CI 1.00;1.59), lower total cholesterol levels (OR 0.87, 95% CI 0.78;0.98), and stroke (OR 2.12, 95% CI 1.40;3.19) were independently related to MCI (Table 2). Male gender and *APOE*-ε4 carriership were only related to amnestic MCI, whereas older age and lower total cholesterol levels were only related to non-amnestic MCI.

Table 1. Characteristics of the study population

	Examinations at baseline 2002-2005		Examinations before baseline 1997-2001	
	No MCI N=3,781	MCI N=417	No MCI[a] N=3,730	MCI[a] N=401
Age, years	71.5 (7.1)	73.5 (7.5)	67.1 (7.0)	68.9 (7.4)
Females	58.2%	52.0%	58.1%	51.1%
APOE-ε4 carrier	26.2%	29.7%	26.2%	30.8%
Educational level				
Primary education	16.8%	24.8%	16.4%	23.8%
Lower vocational education	20.2%	22.4%	20.3%	22.5%
Lower secondary education	17.5%	9.2%	17.6%	9.4%
Intermediate vocational education	26.7%	28.2%	26.8%	28.6%
General secondary education	4.6%	1.9%	4.7%	2.0%
Higher vocational education	12.7%	11.4%	12.7%	11.6%
University	1.6%	1.9%	1.6%	2.0%
Waist circumference, cm	93.4 (11.8)	94.6 (12.4)	93.0 (11.5)	94.1 (11.9)
Hypertension	80.6%	83.0%	65.2%	70.9%
Diabetes mellitus	14.1%	19.2%	8.3%	9.6%
Total cholesterol, mmol/L	5.65 (1.00)	5.43 (0.96)	5.84 (0.97)	5.75 (0.90)
HDL-cholesterol, mmol/L	1.46 (0.40)	1.41 (0.40)	1.40 (0.39)	1.32 (0.36)
Smoking				
Former	55.1%	55.6%	50.4%	50.6%
Current	15.1%	17.5%	19.0%	23.4%
Stroke	3.4%	8.2%	2.0%	5.0%
MRI markers[b]				
Total brain volume, mL	923.9 (89.6)	906.3 (119.9)	NA	NA
Hippocampal volume, mL	3.0 (0.3)	3.0 (0.4)	NA	NA
White matter lesions, mL	3.5 (2.0-6.5)	4.5 (2.6-12.4)	NA	NA
Fractional anisotropy	0.35 (0.02)	0.34 (0.02)	NA	NA
Mean diffusivity, 10^{-3} mm^2/sec	0.77 (0.05)	0.79 (0.05)	NA	NA
Cerebral microbleeds	20.9%	28.6%	NA	NA
Lacunes	5.7%	16.3%	NA	NA

[a]MCI as assessed at baseline (2002-2005)

[b]MRI was performed in a randomly selected subset (N=682).

Continuous variables are presented as means (standard deviations) and categorical variables as percentages. White matter lesions are presented as median (interquartile range).

Abbreviations: MCI=mild cognitive impairment; N=number of participants; *APOE*=apolipoprotein E; HDL=high-density lipoprotein; MRI=magnetic resonance imaging; NA=not applicable.

Table 2. Associations between risk factors of dementia and MCI at baseline (cross-sectional)

	MCI	Amnestic MCI	Non-amnestic MCI
	Odds ratio (95% CI) n/N 389/3,541	**Odds ratio (95% CI) n/N 154/3,541**	**Odds ratio (95% CI) n/N 235/3,541**
Age, per 5 years	1.20 (1.11;1.29)	1.04 (0.92;1.17)	1.30 (1.19;1.43)
Females	0.91 (0.70;1.17)	0.66 (0.45;0.98)	1.11 (0.80;1.54)
APOE-ε4 carrier	1.26 (1.00;1.59)	1.43 (1.01;2.02)	1.17 (0.86;1.58)
Waist circumference, per SD	1.04 (0.92;1.18)	1.03 (0.84;1.25)	1.05 (0.90;1.24)
Hypertension	0.94 (0.70;1.25)	1.03 (0.66;1.59)	0.88 (0.61;1.28)
Diabetes mellitus	1.24 (0.93;1.65)	1.44 (0.94;2.20)	1.11 (0.77;1.59)
Total cholesterol, per SD	0.87 (0.78;0.98)	0.94 (0.79;1.11)	0.84 (0.73;0.97)
HDL-cholesterol, per SD	0.94 (0.83;1.06)	1.02 (0.84;1.23)	0.88 (0.75;1.04)
Smoking			
Former	0.96 (0.74;1.25)	1.08 (0.71;1.66)	0.90 (0.65;1.25)
Current	1.21 (0.86;1.70)	1.37 (0.81;2.31)	1.13 (0.73;1.74)
Stroke	2.12 (1.40;3.19)	2.68 (1.51;4.76)	1.78 (1.04;3.03)

Values represent odds ratios and 95% confidence intervals, adjusted for all other risk factors.
Abbreviations: MCI=mild cognitive impairment; CI=confidence interval; n=number of cases; N=number of controls; *APOE*=apolipoprotein E; SD=standard deviation; HDL=high-density lipoprotein.

Older age (OR per 5 year increase in age 1.18, 95% CI 1.09;1.28), *APOE*-ε4 carriership (OR 1.35, 95% CI 1.06;1.72), lower HDL-cholesterol levels (OR 0.86, 95% CI 0.75;0.98), current smoking (OR 1.49, 95% CI 1.06;2.09) and prevalent stroke (OR 2.50, 95% CI 1.48;4.23) were related to MCI when assessed up to 7 years (mean 4.36 years, SD 0.55) prior to MCI diagnosis (Table 3). *APOE*-ε4 carriership and former and current smoking were related to amnestic MCI, whereas older age and lower HDL-cholesterol levels were related to non-amnestic MCI. Out of 682 participants with MRI scanning, 49 screened positive for MCI. Participants with MCI, particularly those with non-amnestic MCI, had larger WML volumes compared to cognitively healthy participants (mean difference in log-transformed WML volume: 0.36, 95% CI 0.05;0.68). People with non-amnestic MCI also had worse microstructural integrity of normal-appearing white matter after adjustments for cardiovascular risk factors (mean difference in mean FA: -0.007, 95% CI -0.014;-0.001, mean difference in mean MD: 0.013, 95% CI 0.001;0.024) (Table 4, model II). As for focal markers of vascular brain pathology, microbleeds were more frequent in people with MCI, however this association was not significant. Lacunes however, were more frequent in participants with MCI, again particularly in those with non-amnestic MCI (age and sex adjusted OR 3.16, 95% CI 0.98;10.19) (Table 4). MRI scanning was performed on average 1.01 years (SD 0.46) after MCI screening. During this time-interval, 12 of 682 participants who underwent MRI were diagnosed with dementia.

Table 3. Associations between risk factors of dementia, assessed 7 years prior, and MCI

	MCI	Amnestic MCI	Non-amnestic MCI
	Odds ratio (95% CI) n/N 348/3,235	**Odds ratio (95% CI) n/N 140/3,235**	**Odds ratio (95% CI) n/N 208/3,235**
Age, per 5 years	1.18 (1.09;1.28)	1.08 (0.95;1.23)	1.25 (1.13;1.38)
Females	0.93 (0.71;1.21)	0.75 (0.50;1.11)	1.08 (0.77;1.51)
APOE-ε4 carrier	1.35 (1.06;1.72)	1.54 (1.08;2.22)	1.23 (0.90;1.69)
Waist circumference, per SD	1.02 (0.90;1.16)	1.02 (0.84;1.24)	1.03 (0.87;1.21)
Hypertension	1.17 (0.90;1.52)	1.00 (0.68;1.45)	1.33 (0.94;1.87)
Diabetes mellitus	1.05 (0.72;1.54)	0.87 (0.46;1.62)	1.16 (0.73;1.84)
Total cholesterol, per SD	0.95 (0.85;1.07)	0.94 (0.78;1.12)	0.96 (0.83;1.11)
HDL-cholesterol, per SD	0.86 (0.75;0.98)	0.95 (0.78;1.16)	0.80 (0.67;0.95)
Smoking			
Former	1.12 (0.84;1.49)	1.66 (1.01;2.73)	0.92 (0.65;1.31)
Current	1.49 (1.06;2.09)	2.45 (1.41;4.24)	1.12 (0.73;1.73)
Stroke	2.50 (1.48;4.23)	2.91 (1.40;6.06)	2.22 (1.14;4.33)

Values represent odds ratios and 95% confidence intervals, adjusted for all other risk factors and additionally for time between measurements and MCI diagnosis.
Abbreviations: MCI=mild cognitive impairment; CI=confidence interval; n=number of cases; N=number of controls; *APOE*=apolipoprotein E; SD=standard deviation; HDL=high-density lipoprotein.

Out of these, 6 were initially screened as having MCI. Excluding participants with dementia at time of MRI scanning did not change our results (data not shown).
During 24,934 person-years of follow-up, 215 participants developed incident dementia, of whom 177 had Alzheimer disease. During 29,096 person-years of follow-up, 827 people died. Participants with MCI had an increased risk of dementia (age and sex adjusted hazard ratio (HR) 3.98, 95% CI 2.97;5.33) (Table 5). The risk of dementia was especially increased in people with amnestic MCI (HR 6.89, 95% CI 4.74;10.01), but was also increased in people with non-amnestic MCI (HR 2.65, 95% CI 1.79;3.92). Results were similar for Alzheimer disease. We found that participants with MCI also had an increased risk of mortality (HR 1.54, 95% CI 1.28;1.85) (Table 5). These results did not change across MCI-subtypes and additional adjustments did not change our results.

Discussion

We found that in the general population, older age, *APOE*-ε4 carriership, lower total cholesterol levels, and prevalent stroke were associated with MCI. Lower HDL-cholesterol levels and current smoking were only related to MCI when assessed up to 7 years prior to MCI screen-

Table 4. Association between MCI and MRI markers of brain pathology (cross-sectional)

	Volumetric measures Mean difference (95% CI)			Microstructural integrity measures Mean difference (95% CI)		Focal measures Odds ratio (95% CI)	
Model I	**Total brain**	**Hippocampus**	**WML**	**FA**	**MD**	**Microbleeds**	**Lacunes**
No MCI	Reference	Reference	Reference	Reference	Reference	Reference	Reference
MCI	-6.66 (-15.61;2.28)	0.01 (-0.07;0.08)	0.34 (0.11;0.58)	-0.003 (-0.008;0.001)	0.007 (-0.001;0.016)	1.42 (0.73;2.75)	2.68 (1.11;6.45)
Amnestic	-8.37 (-21.16;4.42)	0.05 (-0.06;0.15)	0.32 (-0.02;0.65)	-0.001 (-0.007;0.006)	0.003 (-0.009;0.016)	1.55 (0.61;3.95)	2.31 (0.69;7.68)
Non-amnestic	-5.04 (-17.10;7.01)	-0.03 (-0.13;0.07)	0.36 (0.05;0.68)	-0.006 (-0.012;0.001)	0.010 (-0.001;0.022)	1.35 (0.55;3.31)	3.16 (0.98;10.19)
Model II	**Total brain**	**Hippocampus**	**WML**	**FA**	**MD**	**Microbleeds**	**Lacunes**
No MCI	Reference	Reference	Reference	Reference	Reference	Reference	Reference
MCI	-6.37 (-15.31;2.57)	-0.01 (-0.08;0.07)	0.35 (0.11;0.58)	-0.004 (-0.009;0.001)	0.007 (-0.001;0.016)	1.51 (0.77;2.98)	2.55 (0.99;6.54)
Amnestic	-6.78 (-19.57;6.01)	0.04 (-0.07;0.15)	0.21 (-0.13;0.55)	-0.000 (-0.007;0.007)	0.001 (-0.012;0.013)	1.51 (0.58;3.97)	1.77 (0.49;6.36)
Non-amnestic	-5.77 (-17.86;6.32)	-0.03 (-0.13;0.07)	0.46 (0.14;0.78)	-0.007 (-0.014;-0.001)	0.013 (0.001;0.024)	1.55 (0.62;3.89)	3.83 (1.08;13.61)

Model I: adjusted for age and sex.

Model II: adjusted for age, sex, *apolipoprotein E*-ε4 carriership, waist circumference, hypertension, diabetes mellitus, total and high-density lipoprotein cholesterol, and smoking. Model II was a complete case analysis.

Analyses involving volumetric or microstructural integrity measures were additionally adjusted for intracranial volume.

Volumetric measures were expressed in milliliter (mL), FA has no unit, and MD is expressed in 10^{-3} mm^2/sec. Focal measures were expressed as present versus absent.

Abbreviations: MCI=mild cognitive impairment; MRI=magnetic resonance imaging; CI=confidence interval; WML=white matter lesions volume; FA=fractional anisotropy; MD=mean diffusivity.

Table 5. Associations between MCI and risk of dementia, Alzheimer disease, and mortality (longitudinal)

	Dementia		Alzheimer disease		Mortality	
	n/N	Hazard ratio (95% CI)	n/N	Hazard ratio (95% CI)	n/N	Hazard ratio (95% CI)
Model I						
No MCI	149/3,781	Reference	122/3,781	Reference	695/3,781	Reference
MCI	66/417	3.98 (2.97;5.33)	55/417	4.03 (2.92;5.56)	132/417	1.54 (1.28;1.85)
Amnestic MCI	35/163	6.89 (4.74;10.01)	29/163	7.21 (4.77;10.89)	50/163	1.74 (1.30;2.31)
Non-amnestic MCI	31/254	2.65 (1.79;3.92)	26/254	2.69 (1.75;4.12)	82/254	1.44 (1.14;1.81)
Model II						
No MCI	140/3,541	Reference	114/3,541	Reference	653/3,541	Reference
MCI	59/389	3.70 (2.70;5.05)	49/389	3.75 (2.66;5.30)	123/389	1.48 (1.22;1.80)
Amnestic MCI	33/154	6.76 (4.55;10.03)	27/154	7.20 (4.64;11.18)	46/154	1.58 (1.17;2.14)
Non-amnestic MCI	26/235	2.30 (1.50;3.54)	22/235	2.38 (1.49;3.80)	77/235	1.42 (1.12;1.80)

Model I: adjusted for age and sex.

Model II: adjusted for age, sex, *apolipoprotein E*-ε4 carriership, waist circumference, hypertension, diabetes mellitus, total and high-density lipoprotein cholesterol, smoking, stroke, and educational level.

Values represent hazard ratios and 95% confidence intervals.

Abbreviations: n=number of cases; N=number of persons at risk; CI=confidence interval; MCI=mild cognitive impairment.

ing. Compared to cognitively healthy participants, participants with MCI had larger WML volumes, worse microstructural integrity of normal-appearing white matter, and a higher frequency of lacunes. MCI was associated with an increased risk of dementia, Alzheimer disease, and mortality.

Major strengths of our study are its population-based setting, large sample size, and extensive data collection. Some limitations of our study need to be considered. Firstly, the extensive neuropsychological test battery required for the MCI diagnosis was implemented in 2002-2005 (baseline), and therefore we were not able to assess MCI status on the examination rounds prior to baseline. Therefore, it is possible that some people may already have had MCI at the previous examination round. Secondly, the cross-sectional setting in the analyses of risk factors prevented inferring causality. However, the extensive data collection enabled us to investigate determinants both cross-sectionally at baseline and 7 years prior to baseline, overcoming reverse causality in our study. Thirdly, we did not measure visuospatial ability, and could therefore not include this component in our diagnostic criteria for MCI. Finally, MRI scanning was performed on average 1.01 years (SD 0.46) later than the initial screening for MCI, and misclassification of participants may be present. Nonetheless, 90% of our study participants underwent MRI within 1.5 years of MCI screening, and if present this non-differential misclassification would have led to an underestimation of the true association. Also, we repeated the analyses after excluding incident dementia cases, and found that results did not change materially. We found that some determinants of MCI differed over time. Lower HDL-cholesterol levels and current smoking were only related to MCI when assessed up to 7 years prior to MCI screening. There is a possibility that people with a declining cognitive ability change their daily habits, including dietary and smoking habits, which could result in reverse causality in cross-sectional analysis. Another explanation is that these associations indeed differ over time, as has been shown for several risk factors for dementia.[4,40]

In line with previous clinical and population-based studies, we found that people with MCI had larger WML volumes, worse microstructural integrity of normal-appearing white matter, and a higher prevalence of lacunes compared to cognitively healthy participants.[23,41-43] As regional measurements of DTI were not available in our study, we examined DTI measures averaged over the entire normal appearing white matter. For future investigations however, it would be interesting to study regional differences in FA and MD. MCI was not associated with total brain volume, hippocampal volume, and cerebral microbleeds. These imaging markers have been implicated in people with MCI before[44-49], but relatively small sample size hampered our ability to investigate these associations more thoroughly. Also, smaller total brain volume, hippocampal volume and microbleeds may mark more downstream neuropathology and as such would be a better marker for clinical dementia rather than the transitional stage of MCI.[50]

Participants with MCI had an increased risk of dementia and an increased risk of mortality,

independently of several risk factors of dementia. Because of this poorer prognosis, our findings underline the importance of identifying people with MCI.

It is hypothesized that different subtypes of dementia are preceded by different subtypes of MCI. Amnestic MCI is supposed to especially increase the risk of Alzheimer disease, whereas non-amnestic MCI more likely increases the risk of vascular dementia and other dementia subtypes, such as Lewy body dementia and frontotemporal dementia.[51,52] This would suggest that determinants might also differ per subtype of MCI. However, our findings propose that this distinction is not as unambiguous. On the one hand, we found that there are indeed some differences in determinants for amnestic and non-amnestic subtypes; e.g. *APOE*-ε4 carriership and smoking were related to amnestic MCI only and MRI-correlates of vascular damage, such as larger WML load, altered DTI measures, and lacunes, were more strongly related to non-amnestic MCI. On the other hand, we found that people with MCI who converted to dementia, most often converted to Alzheimer disease, regardless of the MCI subtype. Moreover, stroke was related to both subtypes of MCI. Our results therefore suggest that accumulating vascular damage plays a role in both amnestic and non-amnestic MCI. This is consistent with the fact that vascular disease not only plays an important role in vascular dementia, but also in Alzheimer disease.[2-4,17,53,54] Therefore, we propose that timely targeting modifiable vascular risk factors might contribute to the prevention of MCI and dementia. Nonetheless, it should be kept in mind that the cross-sectional setting of our study in the analyses of risk factors prevents us from drawing any conclusions regarding causality.

We found that people with amnestic MCI had a larger risk of dementia than people with non-amnestic MCI. This difference might be a consequence of the definitions of the MCI subtypes. Study participants with amnestic MCI may have experienced difficulties on other cognitive domains besides memory alone, while participants with non-amnestic MCI per definition did not experience any memory problems. Hence, people with amnestic MCI may have been cognitively more impaired than people with non-amnestic MCI.

In conclusion, in our population-based study we found that several vascular risk factors and MRI-correlates of cerebrovascular disease were associated with MCI. People with MCI had an increased risk of dementia, Alzheimer disease, and mortality.

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Supplementary table and figure

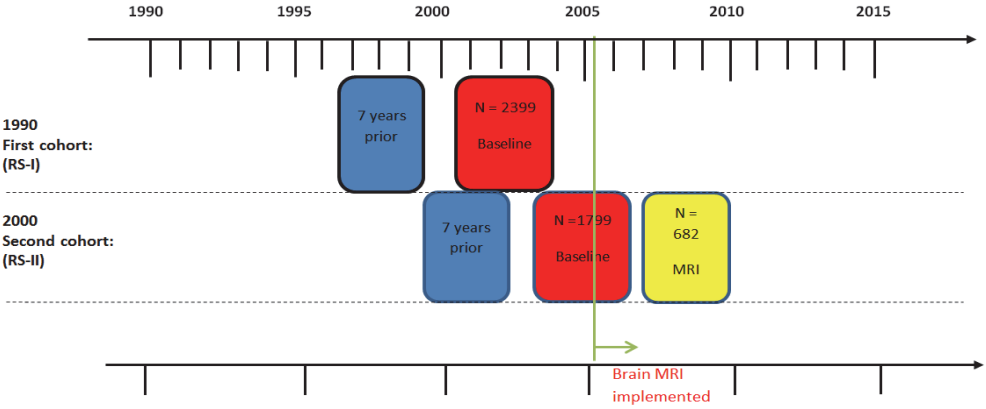
Supplementary Table 1. Characteristics of the included and excluded participants

	Examinations at baseline 2002-2005		Examinations before baseline 1997-2001	
	Included in analysis N=3,930	Excluded from analysis N=268	Included in analysis N=3,583	Excluded from analysis N=615
Age, years	71.8 (7.2)	71.2 (7.2)	67.3 (7.0)	67.5 (7.0)
Females	57.0%	66.0%[a]	56.3%	64.4%[a]
APOE-ε4 carrier	26.5%	26.3%	26.9%	23.9%
Waist circumference, cm	93.5 (11.8)	94.5 (12.8)[a]	93.1 (11.5)	93.0 (12.3)
Hypertension	80.4%	86.9%[a]	64.9%	72.1%[a]
Diabetes mellitus	14.4%	17.9%	8.6%	7.5%
Cholesterol, mmol/L	5.62 (0.99)	5.79 (1.03)	5.84 (0.96)	5.76 (0.98)[a]
HDL-cholesterol, mmol/L	1.45 (0.40)	1.45 (0.40)	1.40 (0.39)	1.38 (0.36)
Smoking				
Former	55.5%	50.0%	51.2%	44.8%
Current	15.2%	18.3%	19.0%	22.7%
Stroke	3.9%	3.0%	2.3%	1.6%

[a] Significantly different (p<0.05) between included participants and excluded participants, after sex and age adjustment – if applicable. Participants excluded from the analysis missed at least one value of the determinants mentioned in the table.

Abbreviations: N=number of participants; *APOE*=apolipoprotein E; HDL=high-density lipoprotein.

Supplementary figure 1. Assessment of determinants, MCI, and MRI examination



In red: baseline measurement of determinants and MCI assessed in 2002-2005. In blue: measurement of determinants assessed in the examination round 7 years prior to baseline. In yellow: a random subset of 682 persons with MCI screening at baseline and brain MRI examination performed on average 1.01 years after baseline (2005 onwards). Abbreviations: MCI=mild cognitive impairment; MRI=magnetic resonance imaging; RS=Rotterdam Study.

Chapter 3

Cardiovascular and metabolic factors

Chapter 3.1

Atrial fibrillation and the risk of dementia

Renée F.A.G. de Bruijn, Jan Heeringa, Frank J. Wolters, Oscar H. Franco, Bruno H.C. Stricker, Albert Hofman, Peter J. Koudstaal, M. Arfan Ikram

Submitted

Abstract

Background: Atrial fibrillation and dementia are common diseases in the elderly. Atrial fibrillation may lead to stroke or chronic cerebral hypoperfusion, which both increase the risk of dementia. As such, it has been suggested that atrial fibrillation is a risk factor of dementia. However, longitudinal studies thus far have shown inconsistent results. We examined whether atrial fibrillation is related to an increased risk of dementia over 20 years of follow-up.

Methods: We assessed prevalent and incident atrial fibrillation in 6,514 participants (mean age 68 years, 58.8% female) of the prospective, population-based Rotterdam Study. The study started in 1990 and follow-up for incident atrial fibrillation and incident dementia was complete until February 4th, 2010. We analyzed separate associations of prevalent atrial fibrillation and incident atrial fibrillation with dementia. Additionally, we censored for stroke and stratified by age. Furthermore, we investigated whether the association between incident atrial fibrillation and dementia differed according to the duration of atrial fibrillation.

Results: At baseline, 318 participants had prevalent atrial fibrillation and during 81,483 person-years of follow-up 994 participants developed incident dementia. Prevalent atrial fibrillation was related to an increased risk of dementia, even after adjustments for cardiovascular risk (HR 1.33, 95% CI 1.02;1.73). During 79,003 person-years of follow-up 723 participants developed incident atrial fibrillation and 932 incident dementia. Incident atrial fibrillation was only borderline associated to an increased risk of dementia (HR 1.23, 95% CI 0.98;1.56). Interestingly, we found that this association was confined to participants below 67 years of age (HR <67 years 1.81, 95% CI 1.11;2.94 versus HR >67 years 1.12, 95% CI 0.85;1.46). Furthermore, we found that the risk of dementia in the younger participants increased gradually with longer duration of atrial fibrillation.

Conclusions: Atrial fibrillation is related to an increased risk of dementia. This association differed with age and was strongest for younger participants who suffered the longest from atrial fibrillation. Future studies should investigate whether optimal treatment of atrial fibrillation can prevent or postpone dementia.

Introduction

Dementia is one of the most common neurological disorders in the elderly. Worldwide, around 40 million people are currently suffering from dementia and this number is expected to increase due to aging of the population.[1] Although the etiology of dementia is largely unknown, there is abundant evidence that cardiovascular disease and its risk factors are involved.[2] Atrial fibrillation is a very common cardiovascular disease in the elderly and its incidence increases with age. Atrial fibrillation might be related to dementia via various pathways.[3,4] First, the most feared complication of atrial fibrillation is stroke, a well-known risk factor of dementia. Hence, stroke might the underlying mechanism between atrial fibrillation and dementia. Second, lower cardiac output in atrial fibrillation leads to chronic cerebral hypoperfusion, which in turn causes damage to nerve cells. Third, a non-causal explanation is shared etiology, since atrial fibrillation and dementia share many risk factors.

Within the Rotterdam Study, we previously showed that atrial fibrillation is more prevalent in people with dementia.[5] However, the cross-sectional design of this study prevented us from drawing conclusions regarding causal inference. Since then, several longitudinal studies have investigated the association between atrial fibrillation and incident dementia, although results have been inconsistent: some studies found that atrial fibrillation increased the risk of cognitive decline or dementia, whereas others found no association.[6-17] These inconsistent findings might be due to the large methodological variation across studies. For example, some studies had relatively small samples or short follow-up periods, which might have limited the power of these analyses. Additionally, the age of the participants between studies differed substantially as did the procedures to assess atrial fibrillation and dementia. Therefore, large, population-based studies with considerable follow-up time are necessary to confirm whether atrial fibrillation is related to an increased risk of dementia.

Within the Rotterdam Study, we investigated the association between atrial fibrillation and dementia over a follow-up period of 20 years. We assessed the associations between prevalent atrial fibrillation and dementia as well as incident atrial fibrillation and dementia. Furthermore, we evaluated whether these associations were independent of stroke and differed according to age.

Materials and methods

Setting and study population

This study was embedded within the Rotterdam Study, a prospective population-based cohort study that aims to assess the occurrence and risk factors of chronic diseases in the elderly. The Rotterdam Study started in 1990 among inhabitants of 55 years and older residing in

Ommoord, a district of Rotterdam, the Netherlands. Of the 10,215 invited inhabitants, 7,983 agreed to participate in the baseline examinations. Follow-up examinations take place every three to four years. Details regarding the objective and design of the Rotterdam Study are described elsewhere.[18]

For this study, we excluded 455 participants because they were not properly screened for dementia, 482 participants because they had prevalent dementia, and 43 for lack of follow-up information on the dementia diagnosis. Additionally, 489 participants were excluded due to missing data on atrial fibrillation. Finally, 6,514 participants were included in the analyses with prevalent atrial fibrillation. For the analyses with incident atrial fibrillation, we excluded the 318 participants with prevalent atrial fibrillation and two due to lack of follow-up data on incident atrial fibrillation, resulting in a total of 6,194 participants for these analyses. When additionally censoring for stroke, we excluded 195 participants who had a stroke at baseline and five participants for lack of follow-up information on incident stroke. In the analyses with incident atrial fibrillation, only 175 participants with prevalent stroke were excluded because 25 were already left out since they had prevalent atrial fibrillation.

The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.

Assessment of atrial fibrillation

Prevalent and incident atrial fibrillation was assessed using three methods.[19] Electrocardiograms (ECG) were performed at baseline and each follow-up examination. These ECGs were stored digitally and analyzed by the Modula ECG Analyses-System (MEANS), which has a high sensitivity and specificity in coding cardiac arrhythmias.[20,21] To verify the diagnosis of atrial fibrillation, every ECG with a diagnosis of rhythm disorder was coded independently by two research physicians who were blinded to the MEANS diagnosis. In case of disagreement between the research physicians, a cardiologist decided on the final diagnosis. In addition, information was obtained from general practitioners and medical specialists. Lastly, information of all hospital discharges from a Nationwide Medical Registry (LMR) was collected. The date of incident atrial fibrillation was defined as the date of first occurrence of symptoms with subsequent ECG verification. We did not differentiate between atrial fibrillation and atrial flutter when identifying cases, since these conditions are very similar with regard to risk factors and consequences.[22,23] Follow-up for incident atrial fibrillation was complete until February 4th, 2010.

Assessment of dementia

Participants were screened for dementia at baseline and follow-up examinations using a

three-step protocol. Screening was done using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level.[24,25] Screen-positives (MMSE<26 or GMS organic level>0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[26] Participants suspected of having dementia, underwent further neuropsychological testing if necessary. Additionally, for people not visiting the research center, the total cohort was continuously monitored for dementia through computerized linkage of the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. When clinical neuroimaging was required and available, it was used for decision making on the diagnosis. A consensus panel, led by a neurologist, decided on the final diagnosis in accordance with internationally accepted standard criteria.[27,28] Follow-up for dementia was complete until February 4th, 2010.

Selection and measurement of risk factors

Weight and height were measured at the research center visit and body mass index (BMI) was calculated as weight in kilograms divided by length in meters squared. Blood pressure was measured in sitting position on the right arm and calculated as the average of two measurements using a random-zero sphygmomanometer. Diabetes mellitus was defined as a fasting serum glucose level ≥7.0 mmol/L, non-fasting serum glucose level ≥11.1 mmol/L, or use of anti-diabetic medication. Serum glucose, total cholesterol, and high-density lipoprotein (HDL)-cholesterol levels were acquired by an automated enzymatic procedure (Boehringer Mannheim System). Information on blood pressure lowering medication, lipid lowering medication, educational level, and smoking habits were assessed by interview. Information on ever use of oral anticoagulant medication was collected using all filled prescriptions from the pharmacies for all participants. Smoking habits were categorized as current, former, and never smoking. Information on *apolipoprotein E (APOE)*-genotype, coded as one or two ε4 alleles, was obtained using polymerase chain reaction on coded DNA samples. History of stroke, coronary heart disease (myocardial infarction or revascularization procedure) and heart failure was evaluated using home interviews and confirmed by reviewing medical records.[29,30] Incident stroke was assessed continuously through automatic linkage of general practitioners' medical records with the study database. In addition, general practitioners' medical records of participants who moved out of the Ommoord district and nursing home physicians' medical records were checked on a regular basis. Of all potential strokes, information from general practitioners and hospital discharge letters were collected and reviewed by research physicians. An experienced neurologist verified the stroke diagnoses.[29]

Statistical analyses

Differences between baseline characteristics of people with and without prevalent atrial fibrillation were studied using logistic regression models, adjusting for age and sex where appropriate. We assessed the association between atrial fibrillation and incident dementia using Cox proportional hazards models. The underlying time scale in these models was the follow-up time. Follow-up started on the date participants entered the Rotterdam Study. Participants were censored at date of death, date of loss to follow-up, or end of the study period, defined as the last date of follow-up or February 4th, 2010, whichever came first. In sensitivity analyses, we additionally censored follow-up time at date of stroke, if a stroke occurred prior to the end of follow-up. We investigated both prevalent and incident atrial fibrillation in separate analyses. Prevalent atrial fibrillation was entered dichotomously into the models whereas incident atrial fibrillation was entered into the models as time-varying variable. In the latter analyses, participants with prevalent atrial fibrillation were excluded as we were not aware of the time-span they had been suffering from atrial fibrillation. The basic model (model I) was adjusted for age and sex. The extended model (model II) was additionally adjusted for diabetes, smoking, total cholesterol, HDL-cholesterol, use of lipid lowering medication, systolic and diastolic blood pressure, use of blood pressure lowering medication, body mass index, educational level, coronary heart disease, heart failure, *APOE-ε4* carrier status, and for ever use of oral anticoagulant medication.

We studied potential effect modification by age using an interaction term and by stratifying analyses at the median age. Additionally, we investigated whether the association between atrial fibrillation and dementia differed according to duration of atrial fibrillation by stratifying follow-up time since onset of atrial fibrillation into three categories: >0 and ≤6 years, >6 and ≤12 years, >12 years until the end of follow-up. These cut-offs were chosen to equally divide the follow-up time for atrial fibrillation, which was highest at 18.8 years.

Missing data on covariates (less than 4.0%) were imputed using multiple imputations. Analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, USA).

Results

Table 1 represents the baseline characteristics of the study population. At baseline, 318 participants had atrial fibrillation. Those participants were older, more often used blood pressure lowering medication, had lower HDL-cholesterol levels, and more often suffered from diabetes mellitus, coronary heart disease, and heart failure than participants without prevalent atrial fibrillation. Conversely, people without prevalent atrial fibrillation had higher total cholesterol levels. In the analyses with prevalent atrial fibrillation, 994 participants developed incident dementia (of whom 787 Alzheimer disease) during 81,483 person-years of follow

Table 1. Baseline characteristics

	No prevalent atrial fibrillation N=6,196	Prevalent atrial fibrillation N=318	P-value for difference
Age, years	68.3 (8.5)	75.7 (8.1)	<0.001
Sex, female	3,678 (59.4)	161 (50.6)	<0.001
Body mass index, kg/m^2	26.3 (3.7)	26.0 (3.6)	0.48
Systolic blood pressure, mmHg	139 (22)	142 (25)	0.40
Diastolic blood pressure, mmHg	74 (11)	73 (13)	0.52
Blood pressure lowering medication	1,367 (22.1)	109 (34.9)	<0.001
Diabetes mellitus	609 (9.9)	64 (20.1)	<0.001
Total cholesterol, mmol/L	6.7 (1.2)	6.2 (1.2)	<0.001
HDL-cholesterol, mmol/L	1.4 (0.4)	1.2 (0.3)	<0.001
Lipid lowering medication	151 (2.4)	9 (2.8)	0.09
Smoking			
Former	2,548 (42.2)	136 (44.3)	0.74
Current	1,429 (23.3)	56 (18.2)	0.35
Apolipoprotein E-ε4 carrier	1,646 (27.8)	82 (26.5)	0.95
Educational level			
Primary education	2,235 (36.6)	126 (40.8)	Reference
Lower vocational education	1,006 (16.5)	51 (16.5)	0.08
Lower secondary education	673 (11.0)	29 (9.4)	0.68
Intermediate vocational education	1,463 (24.0)	80 (25.9)	0.09
General secondary education	198 (3.2)	6 (1.9)	0.54
Higher vocational education	470 (7.7)	16 (5.2)	0.63
University	64 (1.0)	1 (0.3)	0.32
Ever use of oral anticoagulant medication	1,386 (22.4)	87 (27.4)	<0.001
Coronary heart disease	468 (7.9)	53 (18.0)	<0.001
Heart failure	152 (2.5)	58 (18.8)	<0.001

Data are presented as means (standard deviations) or numbers (percentages). Percentages are calculated without missing data. Differences are calculated using logistic regression models.
Abbreviations: N=number of participants; HDL=high-density lipoprotein.

up. In the analyses with incident atrial fibrillation, 723 participants developed incident atrial fibrillation and 932 dementia (of whom 741 Alzheimer disease) during 79,003 person-years of follow-up.

We found that people with prevalent atrial fibrillation had an increased risk of dementia (age and sex adjusted hazard ratio (HR) 1.34, 95% confidence interval (CI) 1.03;1.74). Results were similar after additional adjustments. In contrast, we only found a borderline association be-

Table 2. Atrial fibrillation and the risk of dementia

	Dementia Hazard ratio (95% CI)			Alzheimer disease Hazard ratio (95% CI)		
	n/N	Model I	Model II	n/N	Model I	Model II
Including stroke						
Prevalent atrial fibrillation	994/6,514	1.34 (1.03;1.74)	1.33 (1.02;1.73)	787/6,514	1.30 (0.96;1.75)	1.29 (0.95;1.75)
Incident atrial fibrillation	932/6,194	1.13 (0.90;1.41)	1.23 (0.98;1.56)	741/6,194	1.09 (0.85;1.40)	1.18 (0.91;1.54)
Censored for stroke						
Prevalent atrial fibrillation	844/6,314	1.35 (1.01;1.81)	1.33 (0.99;1.78)	705/6,314	1.31 (0.94; 1.81)	1.28 (0.93;1.78)
Incident atrial fibrillation	793/6,019	1.14 (0.89;1.49)	1.24 (0.96;1.61)	665/6,019	1.08 (0.82;1.42)	1.15 (0.87;1.54)

Model I: adjusted for age and sex.

Model II: additionally adjusted for diabetes, smoking, total cholesterol, HDL-cholesterol, use of lipid lowering medication, systolic and diastolic blood pressure, use of blood pressure lowering medication, body mass index, educational level, ever use of oral anticoagulant medication, coronary heart disease, heart failure , and *apolipoprotein E*-ε4 carrier status.

Abbreviations: CI=confidence interval; n=number of cases; N=number of participants; HDL=high-density lipoprotein.

tween incident atrial fibrillation and dementia after additional adjustments (HR 1.23, 95% CI 0.98;1.56). For both prevalent and incident atrial fibrillation, associations slightly attenuated when we separately investigated Alzheimer disease. Censoring for stroke did not materially change the results (Table 2).

We found that the association of both prevalent and incident atrial fibrillation with dementia differed with age (p-values for interaction 0.04 and 0.02, respectively) (Table 3). When we

Table 3. Atrial fibrillation and the risk of dementia, stratified for age at median

	Dementia Hazard ratio (95% CI)			
	n/N	<67 years	n/N	≥67 years
Prevalent atrial fibrillation	213/3,096	1.91 (0.85;4.26)	781/3,418	1.28 (0.97;1.70)
Incident atrial fibrillation	206/3,049	1.81 (1.11;2.94)	726/3,145	1.12 (0.85;1.46)

Model adjusted for age, sex, diabetes, smoking, total cholesterol, HDL-cholesterol, use of lipid lowering medication, systolic and diastolic blood pressure, use of blood pressure lowering medication, body mass index, educational level, ever use of oral anticoagulant medication, coronary heart disease, heart failure , and *apolipoprotein E*-ε4 carrier status.

Abbreviations: CI=confidence interval; n=number of cases; N=number of participants; HDL=high-density lipoprotein.

stratified analyses at the median age of the population, we found that the association between atrial fibrillation and dementia was strongest in the younger participants. In younger participants the association of incident atrial fibrillation with dementia reached statistical significance (HR 1.81, 95% CI 1.11;2.94) (Table 3). Furthermore, among younger participants we found that the risk of dementia was higher when the duration of atrial fibrillation increased,

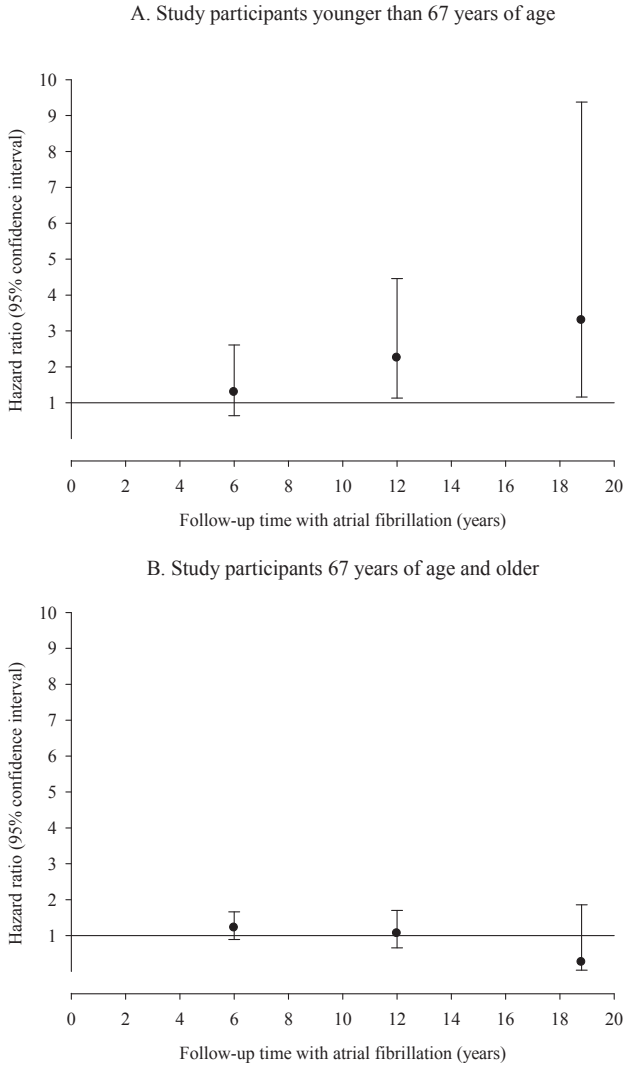


Figure 1. Hazard ratios (95% confidence intervals) for dementia per category of follow-up time suffered from atrial fibrillation.

Cut-offs for categories: >0 and ≤6 years, >6 and ≤12 years, >12 years until the end of follow-up.

but not among older participants (Figure 1). For follow-up time exceeding 12 years, the HR in participants below the median age was 3.30 (1.16;9.38), whereas in participants above the median age it was 0.25 (0.04;1.86).

Discussion

Within the population-based Rotterdam Study, we found that people with atrial fibrillation had an increased risk of dementia. This association was similar for Alzheimer disease and independent of stroke. The risk of dementia was highest in younger participants. Furthermore, in these participants, we found that risk of dementia was higher when they suffered longer from atrial fibrillation.

Before these results can be interpreted, several methodological considerations need to be discussed. Strengths of the study are the population-based and prospective design, the relatively long follow-up period, and the meticulous case-finding procedure for dementia. Additionally, we assessed both the associations of prevalent atrial fibrillation with dementia as well as incident atrial fibrillation with dementia. There are also several limitations of our study. First, we could not distinguish between persistent and paroxysmal atrial fibrillation. Second, atrial fibrillation can occur without symptoms, and although repeated ECG-measurements were performed at the research center, we might have missed some participants with asymptomatic atrial fibrillation. Third, with the exception of oral anticoagulant use, other potential confounders were assessed only at baseline. This might have led to misclassification of these confounders, which could have influenced our results, particularly those with incident atrial fibrillation. Fourth, we did not have information regarding treatment of atrial fibrillation. It is possible that the risk of dementia for people with atrial fibrillation attenuates after successful treatment. Lastly, the Rotterdam Study population is a relatively homogeneous population, consisting mostly of Caucasians that live in a middle income district, which limits the generalizability of results.

Similar to our results, several studies found that people with atrial fibrillation had an increased risk of cognitive decline or dementia.[6-10,17] Conversely, others were not able to demonstrate this link.[11-16] Methodological variability is a likely explanation for these inconsistent findings. For example, smaller sample size or shorter follow-up periods might have hampered the possibility to find associations in some studies. Additionally, other studies relied on registry-based data only, which increases the possibility of misclassification. Another important difference across studies was the age of the study participants. Participants tended to be older in the studies that did not find an association. In line with this, we found that incident atrial fibrillation was a risk factor of dementia in younger participants only. Since dementia gradually develops over years, atrial fibrillation probably needs to develop at a younger age to contribute

to the neuropathology responsible for dementia. Similarly, associations of other dementia risk factors, such as hypertension, hypercholesterolemia, and obesity also appear to differ with age; these factors are risk factors of dementia only when assessed earlier in life.[31-33] In line with this reasoning, if atrial fibrillation is a causal factor in the etiology of dementia, one would expect that the longer a person suffers from this condition, the higher the risk of dementia would be. Indeed, we demonstrated that the risk of dementia was highest for people who suffered the longest from atrial fibrillation. However, this dose-response relation was only present in younger participants. In contrast to our findings, a recent study concluded that the presence of atrial fibrillation at midlife was not a risk factor of dementia, whereas late-life atrial fibrillation was.[6] However, survival bias might have influenced these results, because only participants who survived until a re-examination in late-life were included in the study.

There are several mechanisms that could explain the relation between atrial fibrillation and dementia.[3,4] First, the association between atrial fibrillation might be caused via stroke. Although our results remained similar after censoring for stroke, it remains possible that asymptomatic strokes explain the link between atrial fibrillation and dementia. Such asymptomatic strokes are often lacunes, which are related to an increased risk of dementia.[34] Second, the brain is very vulnerable to changes in blood flow. Hence, cerebral hypoperfusion due to lower cardiac output in atrial fibrillation could cause damage to nerve cells.[35] Third, a non-causal explanation is shared etiology as atrial fibrillation and dementia share many risk factors like hypertension, diabetes mellitus, and hypercholesterolemia. Our results do not strongly support the latter explanation, since they did not attenuate after adjustments for cardiovascular risk factors. Furthermore, we found a dose-response relation between atrial fibrillation and the risk of dementia in younger participants, which is also more suggestive of a causal association. However, the exact mechanism underlying the link between atrial fibrillation and dementia should be further investigated.

Our finding, that atrial fibrillation is a risk factor of dementia has important clinical implications. Given that atrial fibrillation has treatment options, the onset of dementia could be prevented or postponed by optimal treatment.[36] The primary treatment option to prevent stroke is anticoagulant medication. Although we adjusted for ever use of anticoagulant medication, we were not able to adjust for the effectiveness of the treatment. This is of importance, since oral anticoagulant drugs have a narrow therapeutic range. Previous studies have shown that in patients on anticoagulant drugs, time outside the therapeutic range is associated with an increased risk of dementia.[37] Furthermore, we did not have any information on treatment options for atrial fibrillation such as anti-arrhythmic medication, cardioversion, or catheter ablation. A previous study suggested that patients with atrial fibrillation who underwent catheter ablation had a lower risk of dementia than patients who were not treated.[38] However, this was a registry-based study that had a relatively short follow-up period. Future studies

should investigate whether optimal treatment of atrial fibrillation can indeed prevent or delay the onset of dementia.
In conclusion, we found that atrial fibrillation was related to an increased risk of dementia. Furthermore, there appeared to be a dose-response relation; the longer people suffered from atrial fibrillation, the higher their risk of dementia. However, this association was confined to the younger part of our population only. Future studies should determine whether optimal treatment op atrial fibrillation can prevent of postpone dementia.

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Chapter 3.2

Subclinical cardiac dysfunction increases the risk of stroke and dementia in the elderly

Renée F.A.G. de Bruijn*, Marileen L.P. Portegies*, Maarten J.G. Leening, Michiel J. Bos, Albert Hofman, Aad van der Lugt, Wiro J. Niessen, Meike W. Vernooij, Oscar H. Franco, Peter J. Koudstaal, M. Arfan Ikram

* These authors contributed equally to the manuscript

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Abstract

Background: Clinical heart disease has often been associated with stroke and dementia. However, the relation between subclinical cardiac dysfunction and stroke and dementia is unknown. We investigated the association between cardiac function and the risk of stroke and dementia in elderly free of clinical cardiac disease. Additionally, we investigated the relation between cardiac function and MRI-markers of subclinical cerebrovascular disease.
Methods: This study was conducted within the population-based Rotterdam Study. A total of 3,291 participants (60.8% female, age-range 58-98 years) free of coronary heart disease, heart failure, atrial fibrillation, stroke, and dementia, underwent echocardiography in 2002-2005 to measure cardiac function. Follow-up finished in 2012. In 2005-2006, a random subset of 577 stroke-free non-demented people underwent brain MRI on which infarcts and white matter lesion volume were assessed.
Results: During 21,785 person-years of follow-up 164 people suffered a stroke and during 19,462 person-years of follow-up 208 people developed dementia. Measures of better diastolic function, such as higher E/A-ratio, were associated with a lower risk of stroke (HR 0.82, 95% CI 0.69;0.98) and dementia (HR 0.82, 95% CI 0.70;0.96). Better systolic function, measured as higher fractional shortening, was only associated with a lower risk of stroke (HR 0.84, 95% CI 0.72;0.98). Better diastolic function was related to a lower prevalence of silent infarcts on MRI, especially lacunar infarcts.
Conclusions: In elderly free of clinical cardiac disease, worse diastolic function is associated with clinical stroke, dementia, and silent infarcts on MRI, whereas worse systolic function is related only to clinical stroke. These findings can form the basis for future research on the utility of cardiac function as potential intervention target for prevention of neurological diseases.

Introduction

Stroke and dementia are major neurological diseases in the elderly.[1,2] Cardiovascular risk factors play a role in the etiology of both stroke and dementia, including Alzheimer disease (AD).[1,3] Additionally, clinical cardiac diseases, such as heart failure,[4,5] atrial fibrillation,[6,7] and coronary heart disease[8,9] have been associated with stroke, dementia, AD, and with subclinical cerebrovascular damage, such as silent infarcts[10] and white matter lesions.[11] Cerebrovascular damage accumulates slowly before manifesting as clinical event, and silent brain infarcts and white matter lesions indicate an increased risk of stroke and dementia.[12-14]

On the one hand, shared etiology might explain the link between cardiac and neurological diseases. On the other hand, cardiac disease might be causally related to stroke and dementia via thrombus formation or hypoperfusion.

In the general elderly population, cardiac function is often impaired in the absence of clinical cardiac disease.[15-17] Subclinical cardiac dysfunction has been associated with an increased risk of clinical cardiac events and mortality.[17-19] However, the longitudinal association between subclinical cardiac dysfunction and major neurological outcomes remains unclear. Relating echocardiographic markers of subclinical cardiac dysfunction to stroke and dementia is important for possible preventive strategies.

We investigated whether cardiac function is associated with the risk of stroke and dementia in people without clinical cardiac disease. Additionally, we investigated whether cardiac function is related to magnetic resonance imaging (MRI)-markers of subclinical cerebrovascular disease.

Materials and methods

Setting and study population

This study was embedded within the Rotterdam Study, a prospective population-based cohort study among people aged 55 years and older residing in Ommoord, a district of Rotterdam, the Netherlands.[20] The study started in 1990 with 7,983 participants and was extended in 2000 with 3,011 people. Follow-up examinations take place every 3 to 4 years.[20]

For the current study, data on echocardiography were collected in 2002-2005, because echocardiography was only introduced in this round. During this period 3,550 participants of the original cohort attended their fourth examination round and 2,486 participants of the cohort expansion attended their second examination round. Of these participants, 5,395 actually visited the study center and 5,287 underwent echocardiography. Missing echocardiograms were primarily caused by absence of echocardiographers and were random. We excluded participants who had a poor quality echocardiogram (n=233) or missed measurements on the

echocardiogram (n=483). Participants with prevalent heart failure, coronary heart disease, atrial fibrillation, stroke, or dementia (n=765), or missing data on these diseases (n=515) were also excluded. Consequently, 3,291 participants were eligible for analysis. Because MRI-scanning was implemented in 2005,[21] only a random subset of 593 non-demented, stroke-free people had available brain MRI, of which 577 had good quality MRI data. For analyses with white matter lesions, cortical infarcts (n=6) were excluded, as tissue loss and gliosis surrounding cortical infarcts may cause unreliable segmentations. MRI-scanning was performed on average 1.0 (standard deviation (SD) ±0.4) years after echocardiography.

Standard protocol approvals, registrations, and patient consents

The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study and written informed consent was obtained.

Echocardiography

Transthoracic echocardiograms were performed by four trained echocardiographers using commercially available systems (AU3 Partner, Esaote Biomedica, Hallbergmoos, Germany, with a 3.5/2.5MHz transducer, n=1,289; or Acuson Cypress, Siemens, Mountain View, USA, with a 3V2c transducer, n=2,002).

To assess diastolic function, transmitral filling velocities were recorded by pulsed wave Doppler in the apical four-chamber view. The sample volume was placed in the mitral valve orifice near the tips of the leaflets. The early filling velocity occurring with mitral valve opening is the peak E velocity. Peak A velocity is the velocity occurring with contraction of the atrium.[22] Doppler peak E and peak A velocities were averaged over three cycles. Doppler peak E velocity was divided by Doppler peak A velocity to calculate E/A-ratio. The time between the peak E wave and the upper deceleration slope extrapolated to the zero baseline is the early mitral valve deceleration time. Left ventricular diastolic function was categorized,[18] using cut-off points as described.[16,22] Diastolic function was classified as normal (E/A-ratio 0.75-1.50 and deceleration time 150-280ms), impaired relaxation (E/A-ratio <0.75 and deceleration time >280ms), or restrictive (E/A-ratio >1.50 and deceleration time <150ms). If only one abnormal criterion was fulfilled, diastolic function was classified as indeterminate instead of normal.[18]

To assess systolic function, we measured left ventricular end-systolic dimension (LVESD) and left ventricular end diastolic dimension (LVEDD) in the parasternal long axis view using M-mode with 2-dimensional guidance as described previously.[15,18] Fractional shortening was calculated as (LVEDD-LVESD)/LVEDD*100%. Qualitative global systolic function was assessed from the 2-dimensional echocardiogram in four categories: normal, fair, moderate, and poor. Inter-reader and intra-reader variability of the echocardiography measurements were good.[18]

Assessment of stroke

At baseline, history of stroke was assessed using home interviews and confirmed by reviewing medical records. Participants were then continuously followed-up for stroke through automatic linkage of general practitioners' medical records with the study database. Furthermore, general practitioners' medical records of participants who moved out of the Ommoord district and nursing home physicians' medical records were checked on a regular basis.[23] Of all potential strokes, information from general practitioners and hospital discharge letters were collected and reviewed by research physicians. An experienced neurologist verified the stroke diagnoses. Strokes were sub-classified into ischemic or hemorrhagic based on neuroimaging reports. A stroke was classified as unspecified if lacking neuroimaging.[24] Follow-up was complete until January 1st, 2012 for 92.7% of potential person-years.

Assessment of dementia

We used a three-step protocol to screen for dementia at baseline and follow-up examinations. First, participants underwent the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Second, screen-positives (MMSE<26 or GMS organic level>0) underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX). Third, participants suspected of dementia underwent further neuropsychological testing if necessary. Additionally, all participants were continuously monitored for dementia linking the study database to digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. When clinical neuroimaging was required and available, it was used for decision making on the diagnosis. The final diagnosis was determined by a consensus panel, led by a neurologist, in accordance with international criteria.[25,26] Follow-up for incident dementia was complete until January 1st, 2012, for 97.9% of potential person-years.[27]

Brain MRI

Scans were performed on a 1.5T MRI scanner (General Electric Healthcare, Milwaukee, USA) and reviewed by trained research physicians, who were blinded to clinical data. We used fluid-attenuated inversion recovery (FLAIR), proton density weighted, and T1-weighted sequences to identify infarcts. Focal lesions of ≥3mm and <15mm in size with identical signal characteristics as cerebrospinal fluid, and (when located supratentorially) a hyperintense rim on the FLAIR were classified as lacunar infarcts. Infarcts showing involvement of grey matter were classified as cortical infarcts. Because all people undergoing MRI were free of clinical stroke, all infarcts are silent infarcts. White matter lesions were segmented based on the FLAIR using an automated processing algorithm and voxels were summed to yield total white matter lesion volume in ml.[21]

Covariates

Covariates were measured during the same examination round as the echocardiography (2002-2005). Details on assessment of anthropometrics, cardiovascular risk factors (blood pressure, total cholesterol, high-density lipoprotein (HDL)-cholesterol, diabetes mellitus, smoking), and medication use have been described.[28] Heart failure, coronary heart disease (defined as myocardial infarction or coronary revascularization procedure), and atrial fibrillation were assessed through active follow-up and adjudicated using standardized definitions.[23] MMSE was performed at the research center. Information on *apolipoprotein E (APOE)*-genotype, coded as one or two ε4 alleles, was obtained using polymerase chain reaction on coded DNA samples.

Statistical analyses

We examined the association of cardiac function with stroke and dementia using Cox proportional hazards models. Follow-up started at the echocardiography date. We censored participants at date of stroke in analyses with stroke and at date of dementia diagnosis in analyses with dementia. Participants were additionally censored at date of death, date of loss to follow-up, or the end of the study period, defined as the last date of follow-up or January 1st, 2012, whichever came first. We conducted a sensitivity analysis censoring for both stroke and dementia concomitantly in every analysis. We used linear and logistic regression models to investigate the associations of cardiac function with silent infarcts and white matter lesions. White matter lesion volume, mitral valve inflow deceleration time, and E/A-ratio were natural log transformed because of skewed distributions to the right. Fractional shortening was square transformed because of a skewed distribution to the left. Continuous echocardiographic variables were entered per SD increase into the models. Qualitative systolic function and diastolic function were entered categorically into the models. Only ten participants had diastolic dysfunction with a restrictive pattern: therefore we combined diastolic dysfunction with impaired relaxation or a restrictive pattern for the analysis. Only 16 participants had poor left ventricular systolic function, which we therefore combined with moderate systolic function. In sensitivity analyses, we excluded participants (n=66) with moderate or poor systolic function to determine whether associations remained similar in people with a normal left ventricular function. The basic model (model I) was adjusted for age, sex, and type of ultrasonography system. The extended model (model II) was additionally adjusted for cardiovascular risk factors, and for MMSE-score and *APOE-ε4* status in dementia analyses only. To further adjust for residual confounding by shared etiology, we repeated the analyses adjusting for cardiovascular risk factors and MMSE-score assessed at the examination round prior (1997-2001) to our baseline. All analyses with white matter lesion volume were also adjusted for intracranial volume.

We explored potential effect modification by sex, age (stratified at median), blood pressure (stratified at median), and medication use by using interaction terms.
Missing data on covariates (less than 3.6%) were imputed using multiple imputations. Analyses were repeated for ischemic stroke and AD.
Analyses were done using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, USA).

Results

Baseline characteristics are presented in Table 1. Mean age (±SD) was 71.4 (±7.1) years and 60.8% was female. During a mean follow-up of 6.6 (±1.8) years, 164 strokes occurred, of which 117 were ischemic. During a mean follow-up of 5.9 (±1.5) years, 208 people developed dementia, of which 171 AD.
Several measures of diastolic function were associated with the risk of stroke, including mitral valve inflow peak A (hazard ratio (HR) per SD increase 1.27 (95% confidence interval (CI) 1.08;1.48)), E/A-ratio (HR 0.82, 95% CI 0.69;0.98), and mitral valve inflow deceleration time (HR 1.22, 95% CI 1.05;1.41), independently of cardiovascular risk factors. Results were similar for ischemic stroke. For systolic function, fractional shortening (HR 0.84, 95% CI 0.72;0.98) and moderate or poor qualitative systolic function (HR 2.56, 95% CI 1.21;5.43) were associated with the risk of stroke, independently of cardiovascular risk factors. Results attenuated slightly when we investigated ischemic stroke (Table 2).
Worse diastolic function was also related to an increased risk of dementia through mitral valve inflow peak E (HR 0.85, 95% CI 0.75;0.96), E/A-ratio (HR 0.82, 95% CI 0.70;0.96), mitral valve inflow deceleration time (HR 1.16, 95% CI 1.02;1.33), and impaired relaxation or restrictive pattern (HR 1.78, 95% CI 1.08;2.95). Results were similar for AD. We did not find any associations between measurements of systolic function and the risk of dementia or AD (Table 3).
Results were similar after censoring for both stroke and dementia concomitantly or after excluding 66 people with moderate or poor left ventricular systolic function (results not shown). Associations were also similar after adjusting for cardiovascular risk factors and MMSE-score assessed at the examination round prior to our baseline (Supplementary tables 1 and 2).
We did not find a consistent pattern of effect modification by age, sex, blood pressure, or medication use, which was further hampered by smaller sample sizes in the respective strata. The only potential interaction was observed between sex and mitral valve inflow peak E for stroke (HR 0.71, 95% CI 0.53;0.94) in men and HR 1.27, 95% CI 1.07;1.51 in women). For dementia, we found potential interactions between sex and mitral valve deceleration time (HR 1.46, 95% CI 1.16;1.84) in men and HR 1.05, 95% CI 0.89;1.23 in women) and age and mitral valve deceleration time (HR 1.93, 95% CI 1.25;2.99) for <70.4 years and HR 1.11, 95% CI

Table 1. Baseline characteristics

	At risk
	N=3,291
Demographics	
Age, years	71.4 (7.1)
Female	2,001 (60.8%)
Score on MMSE, points	27.6 (2.1)
Cardiovascular risk factors	
Systolic blood pressure, mm Hg	150 (21)
Diastolic blood pressure, mm Hg	80 (11)
Use of blood pressure lowering medication	1,002 (30.8%)
Heart rate, b/min	69 (10)
Total cholesterol, mmol/L	5.7 (0.9)
HDL-cholesterol, mmol/L	1.5 (0.4)
Lipid lowering medication	559 (17.2%)
Diabetes mellitus	434 (13.2%)
Smoking	
Past	1,692 (52.6%)
Current	509 (15.8%)
Body mass index	27.4 (3.9)
APOE-ε4 carrier	850 (26.8%)
Measures of diastolic function	
Mitral valve inflow peak E, m/s	0.65 (0.15)
Mitral valve inflow peak A, m/s	0.77 (0.17)
Mitral valve inflow deceleration time, ms*	208 (184-240)
E/A-ratio*	0.83 (0.71-1.00)
Qualitatively assessed diastolic function	
Normal	2,027 (61.6%)
Indeterminate	1,110 (33.7%)
Impaired relaxation	144 (4.4%)
Restrictive pattern	10 (0.3%)
Measures of systolic function	
Fractional shortening, %*	40.4 (35.6-44.0)
Qualitatively assessed systolic function	
Normal	2,102 (63.9%)
Fair	1,123 (34.1%)
Moderate	50 (1.5%)
Poor	16 (0.5%)

Data are presented as mean (standard deviations) or counts (percentages).

* Median and inter-quartile range because of skewed distribution

Abbreviations: N=number of persons included in study; HDL=high-density lipoprotein; MMSE=Mini-Mental State Examination; *APOE=apolipoprotein E.*

Table 2. Cardiac function and the risk of stroke

	Stroke n/N 164/3,291		Ischemic stroke n/N 117/3,291	
	Model I	Model II	Model I	Model II
Diastolic function				
Quantitative diastolic function				
Mitral valve inflow peak E, per SD	1.11 (0.96;1.29)	1.07 (0.92;1.25)	1.21 (1.02;1.43)	1.15 (0.96;1.38)
Mitral valve inflow peak A, per SD	1.23 (1.06;1.43)	1.27 (1.08;1.48)	1.24 (1.04;1.49)	1.25 (1.04;1.52)
Mitral valve inflow deceleration time*, per SD	1.21 (1.04;1.41)	1.22 (1.05;1.41)	1.18 (0.99;1.42)	1.19 (0.99;1.42)
E/A-ratio*, per SD	0.88 (0.74;1.03)	0.82 (0.69;0.98)	0.95 (0.78;1.15)	0.88 (0.72;1.08)
Qualitative diastolic function				
Normal	Reference	Reference	Reference	Reference
Indeterminate	1.20 (0.86;1.67)	1.27 (0.91;1.78)	1.22 (0.82;1.80)	1.32 (0.89;1.96)
Impaired relaxation or restrictive pattern	1.69 (0.96;2.99)	1.74 (0.98;3.08)	1.58 (0.77;3.25)	1.65 (0.80;3.40)
Systolic function				
Quantitative systolic function				
Fractional shortening, per SD*	0.85 (0.72;0.99)	0.84 (0.72;0.98)	0.88 (0.73;1.06)	0.87 (0.72;1.05)
Qualitative systolic function				
Normal	Reference	Reference	Reference	Reference
Fair	1.29 (0.93;1.80)	1.35 (0.97;1.89)	1.18 (0.80;1.76)	1.25 (0.84;1.87)
Moderate or poor	2.21 (1.05;4.66)	2.56 (1.21;5.43)	1.89 (0.74;4.81)	2.27 (0.89;5.80)

Values are hazard ratios with 95% confidence intervals.
Model I: adjusted for age, sex and type ultrasonography system.
Model II: adjusted for age, sex, type ultrasonography system, body mass index, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid lowering medication, diabetes mellitus, current smoking, past smoking, and heart rate.
* Natural log or square transformed because of skewed distribution.
Abbreviations: n=number of cases; N=number of persons at risk; SD=standard deviation.

0.96;1.27 for >70.4 years). However, such interactions were not observed for other parameters related to diastolic function, which might point towards spurious associations.

Of 577 persons with MRI-data, 37 had a silent infarct, of whom 31 a lacunar infarct. Median white matter lesion volume was 3.42ml (interquartile range 2.17-6.43). Mitral valve inflow deceleration time and E/A-ratio were associated with silent infarcts, especially lacunar infarcts (Table 4). A moderate or poor systolic function was associated with a higher prevalence of silent infarcts, but this group only consisted of four participants resulting in wide CIs (Table 4). We found no associations of cardiac function with white matter lesion volume.

Table 3. Cardiac function and the risk of dementia

	Dementia n/N 208/3,291		Alzheimer disease n/N 171/3,291	
	Model I	Model II	Model I	Model II
Diastolic function				
Quantitative diastolic function				
Mitral valve inflow peak E, per SD	0.90 (0.79;1.04)	0.85 (0.75;0.96)	0.85 (0.72;0.99)	0.79 (0.69;0.91)
Mitral valve inflow peak A, per SD	1.02 (0.89;1.17)	0.92 (0.81;1.05)	1.00 (0.86;1.17)	0.88 (0.76;1.01)
Mitral valve inflow deceleration time*, per SD	1.18 (1.03;1.35)	1.16 (1.02;1.33)	1.25 (1.08;1.44)	1.22 (1.06;1.41)
E/A-ratio*, per SD	0.86 (0.74;0.99)	0.82 (0.70;0.96)	0.83 (0.71;0.97)	0.78 (0.66;0.92)
Qualitative diastolic function				
Normal	Reference	Reference	Reference	Reference
Indeterminate	1.24 (0.92;1.67)	1.41 (1.04;1.92)	1.19 (0.86;1.65)	1.41 (1.01;1.99)
Impaired relaxation or restrictive pattern	1.53 (0.94;2.52)	1.78 (1.08;2.95)	1.43 (0.83;2.48)	1.69 (0.97;2.96)
Systolic function				
Quantitative systolic function				
Fractional shortening, per SD*	0.97 (0.85;1.11)	0.98 (0.85;1.13)	0.95 (0.82;1.11)	0.97 (0.83;1.13)
Qualitative systolic function				
Normal	Reference	Reference	Reference	Reference
Fair	1.13 (0.85;1.51)	1.19 (0.88;1.60)	1.13 (0.82;1.55)	1.19 (0.86;1.66)
Moderate or poor	1.25 (0.57;2.74)	1.21 (0.55;2.68)	1.10 (0.44;2.76)	1.08 (0.43;2.73)

Values are hazard ratios with 95% confidence intervals.
Model I: adjusted for age, sex and type ultrasonography system.
Model II: adjusted for age, sex, type ultrasonography system, body mass index, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid lowering medication, diabetes mellitus, current smoking, past smoking, heart rate, MMSE-score, and *APOE-ε4* carrier status.
* Natural log or square transformed because of skewed distribution.
Abbreviations: n=number of cases; N=number of persons at risk; SD=standard deviation; MMSE=Mini-Mental State Examination; *APOE=apolipoprotein E.*

Discussion

In people free of clinical cardiac disease, worse diastolic function was associated with an increased risk of stroke and dementia, whereas worse systolic function was only associated with a higher risk of stroke. Diastolic function was also related to silent infarcts, especially lacunar infarcts, on MRI.

Patients with overt cardiac disease have an increased risk of stroke and dementia. However, the associations between subclinical cardiac disease and stroke and dementia are unclear. As

Table 4. Cardiac function and MRI-markers (N=577)

	Infarcts		White matter lesion volume*
	Total OR (95%CI)	Lacunar OR (95%CI)	Mean difference (95%CI)
Diastolic function			
Quantitative diastolic function			
Mitral valve inflow peak E, per SD	0.78 (0.51;1.20)	0.68 (0.42;1.09)	0.03 (-0.05;0.10)
Mitral valve inflow peak A, per SD	1.09 (0.71;1.69)	1.16 (0.72;1.88)	0.03 (-0.05;0.11)
Mitral valve inflow deceleration time*, per SD	1.56 (1.04;2.35)	1.70 (1.08;2.69)	-0.01 (-0.08;0.06)
E/A-ratio*, per SD	0.66 (0.41;1.07)	0.52 (0.30;0.90)	0.01 (-0.07;0.09)
Qualitative diastolic function			
Normal	Reference	Reference	Reference
Indeterminate	0.94 (0.38;2.31)	1.07 (0.40;2.83)	-0.05 (-0.21;0.11)
Impaired relaxation or restrictive pattern	4.81 (1.06;21.84)	4.06 (0.68;24.13)	-0.01 (-0.43;0.41)
Systolic function			
Quantitative systolic function			
Fractional shortening, per SD*	0.86 (0.54;1.37)	1.02 (0.61;1.71)	-0.04 (-0.12;0.05)
Qualitative systolic function			
Normal	Reference	Reference	Reference
Fair	1.23 (0.51;2.95)	1.38 (0.54;3.50)	0.01 (-0.16;0.17)
Moderate or poor	43.56 (4.13;460.07)	23.29 (1.22;445.49)	0.57 (-0.31;1.45)

Values are odds ratios or differences in volume with 95% confidence intervals.
Adjusted for age, sex, body mass index, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication, total cholesterol, high-density lipoprotein cholesterol, lipid lowering medication, diabetes mellitus, current smoking, past smoking, and heart rate. White matter lesion volumes were additionally adjusted for intracranial volume.
* Natural log or square transformed because of skewed distribution.
Abbreviations: MRI=magnetic resonance imaging; N=number of persons at risk; OR=odds ratio; CI=confidence interval; SD=standard deviation.

for stroke, other population-based studies have mostly examined the association of cardiac structure and stroke.[19,29,30] However, in patients with heart failure, quality of systolic and diastolic function provides additional information on stroke risk over markers of cardiac structure alone.[31] Little data is available in asymptomatic populations on systolic and diastolic function related to stroke. While diastolic dysfunction was not associated with stroke in the Strong Heart Study, this study population was younger and of different ethnicity than ours.[29] The AGES-Reykjavik study found an association between low E/A-ratio and cerebral infarcts on MRI, supporting our observations, but this was a cross-sectional study that did not examine clinical strokes.[32] For systolic function, the Framingham Heart Study found that

persons with a lower fractional shortening had a higher risk of cardiovascular disease,[33] which is in line with our observations, although they did not separately examine stroke. Hence, our results provide novel evidence that diastolic and systolic dysfunction are both associated with an increased risk of stroke, even within ranges of normal values.
Regarding dementia, the Rotterdam Study previously found a higher prevalence of dementia in people with atrial fibrillation.[7] Findings from several other population-based studies also point towards an association between cardiac dysfunction and dementia. For instance, the Framingham Heart Study investigated the association between cardiac function and low cognitive performance and MRI-markers related to dementia and AD.[34] They found a U-shaped association between left ventricular end systolic function and cognitive performance. Similarly, another study found that diminished cardiac function was related to lower brain volume, an important marker of brain aging.[35] The Cardiovascular Health Study found an association between cardiovascular disease and dementia.[9] However, these studies only investigated people with cardiac disease,[7,9] or were cross-sectional by design.[7,34,35] Our results suggest that diminished diastolic cardiac function is also associated with an increased risk of dementia in people free of cardiac disease. Since results for dementia and AD were similar and even remained stable after censoring for stroke, our study also supports the growing evidence that vascular factors play an important role in the etiology of AD.[36] The question remains why diastolic function, but not systolic function, is related to dementia. Interestingly, we also found diastolic function to be associated with silent infarcts on MRI, which were primarily lacunar infarcts. Cerebral small vessel disease has been suggested to be the underlying link between lacunar infarcts and dementia. Future research should therefore explore whether diastolic function rather than systolic function relates strongest with pathology of the smallest vessels in the brain. Novel imaging techniques, such as arterial spin labeling for brain perfusion and 7T MRI for visualizing small vessels, may play an important role here.

There are several explanations for the relation between cardiac function and the risk of neurologic disease, which are supported equally by our data. Firstly, cardiac dysfunction can lead to cardioembolism,[37] which in turn causes stroke and contributes to the etiology of dementia.[3,38] Secondly, impaired cardiac function may lead to cerebral hypoperfusion. In people with cardiac arrhythmias, hypoperfusion leads to watershed infarction.[39] Furthermore, in patients with heart failure, low ejection fraction has been associated with cognitive impairment.[40] However, low cardiac output is closely related to diminished systolic function, which in our study was not associated with dementia. Lastly, a non-causal explanation is shared etiology, since impaired cardiac function, stroke, and dementia share risk factors.[3,4] Although our results were independent of cardiovascular risk factors, even when assessed up to 7 years prior to baseline, there might still be residual confounding.

Strengths of this study are the population-based design, the long follow-up period, the systematic collection and adjudication of events, and the standardized assessment of risk factors and echocardiographic parameters. A limitation is that categorization of left ventricular diastolic function was based on E/A-ratio and mitral valve deceleration time, and not on the early diastolic longitudinal velocity of the mitral annulus (E').[16,22] We were thus unable to sub-classify diastolic function in approximately one third of our study population. Neither did we systematically measure valvular diseases, which are well-known substrates for cardioembolisms and can affect cardiac function.[38] Another issue is multiple testing, since we tested several diastolic and systolic measures of cardiac function. However, as the associations we found were not completely independent, adjusting for multiple testing might have led to false negative results. Since echocardiography was performed in the fourth examination of the original cohort and the second examination of the extended cohort, survival bias cannot be ruled out. Finally, most participants of the Rotterdam Study are Caucasians and live in a middle income district of Rotterdam, which limits the generalizability of our results.

Our results indicate that in people without clinically overt cardiac disease, impaired diastolic function is associated with the risk of clinical stroke, dementia, and silent infarcts on MRI, whereas impaired systolic function is only associated with the risk of stroke. Future research should determine whether improving cardiac function can prevent stroke and dementia.

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Supplementary tables

Supplementary table 1. Characteristics assessed at the examination prior to baseline

	At risk
	N=3,291
Demographics	
Score on MMSE, points	28.0 (1.7)
Cardiovascular risk factors	
Systolic blood pressure, mm Hg	142 (20)
Diastolic blood pressure, mm Hg	77 (11)
Use of blood pressure lowering medication	634 (20.1%)
Heart rate, b/min	70 (10)
Total cholesterol, mmol/L	5.9 (0.9)
HDL-cholesterol, mmol/L	1.4 (0.4)
Lipid lowering medication	314 (9.9%)
Diabetes mellitus	308 (9.7%)
Smoking	
Past	1,535 (47.7%)
Current	639 (19.9%)
Body mass index	26.8 (3.8)
APOE-ε4 carrier	850 (26.8%)

Data are presented as means (standard deviations) or counts (percentages).
Abbreviations: N=number of people included in study; HDL=high-density lipoprotein; MMSE=Mini-Mental State Examination; *APOE=apolipoprotein E.*

Supplementary table 2. Cardiac function and the risk of stroke and dementia, adjusted for potential confounders assessed at the examination prior to baseline

	Stroke n/N 164/3,291	Dementia n/N 208/3,291
Diastolic function		
Quantitative diastolic function		
Mitral valve inflow peak E, per SD	1.09 (0.94;1.27)	0.89 (0.77;1.02)
Mitral valve inflow peak A, per SD	1.21 (1.04;1.41)	1.03 (0.89;1.18)
Mitral valve inflow deceleration time*, per SD	1.21 (1.04;1.41)	1.16 (1.02;1.32)
E/A-ratio*, per SD	0.88 (0.74;1.03)	0.84 (0.72;0.97)
Qualitative diastolic function		
Normal	Reference	Reference
Indeterminate	1.19 (0.85;1.66)	1.26 (0.93;1.70)
Impaired relaxation or restrictive pattern	1.66 (0.94;2.94)	1.67 (1.02;2.74)
Systolic function		
Quantitative systolic function		
Fractional shortening, per SD*	0.83 (0.71;0.97)	0.97 (0.84;1.11)
Qualitative systolic function		
Normal	Reference	Reference
Fair	1.35 (0.96;1.88)	1.15 (0.86;1.54)
Moderate or poor	2.62 (1.24;5.56)	1.26 (0.57;2.77)

Values are hazard ratios with 95% confidence intervals.

Adjusted for age, sex, type ultrasonography system, body mass index, systolic blood pressure, diastolic blood pressure, use of blood pressure lowering medication, total cholesterol, high-density lipoprotein cholesterol, use of lipid lowering medication, diabetes mellitus, current smoking, past smoking, heart rate, and for MMSE-score and *APOE-ε4* carrier status in dementia analyses only.

* Natural log or square transformed because of skewed distribution.

Abbreviations: n=number of cases; N=number of persons at risk; SD=standard deviation; MMSE=Mini-Mental State Examination; *APOE=apolipoprotein E*.

Chapter 3.3

Atherosclerotic calcification is related to a higher risk of dementia and cognitive decline

Daniel Bos, Meike W. Vernooij, Renée F.A.G. de Bruijn, Peter J. Koudstaal, Albert Hofman, Oscar H. Franco, Aad van der Lugt, M. Arfan Ikram

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Abstract

Background: Longitudinal data on the role of atherosclerosis in different vessel beds in the etiology of cognitive impairment and dementia are scarce and inconsistent.
Methods: In 2003-2006, 2,364 non-demented persons underwent CT of the coronaries, aortic arch, extracranial, and intracranial carotid arteries to quantify atherosclerotic calcification. Participants were followed for incident dementia (n=90) until April 2012. At baseline and follow-up, participants also underwent a cognitive test battery.
Results: Larger calcification volume in all vessels, except the coronaries, was associated with a higher risk of dementia. After adjustment for relevant confounders, extracranial carotid artery calcification remained significantly associated with a higher risk of dementia (HR per SD 1.37, 95% CI 1.05;1.79). Additional analyses for Alzheimer disease only or censoring for stroke showed similar results. Larger calcification volume was also associated with cognitive decline.
Conclusions: Atherosclerosis, in particular in the extracranial carotid arteries, is related to a higher risk of dementia and cognitive decline.

Introduction

Dementia, including Alzheimer disease, is a devastating condition with a huge societal impact, both in terms of patient suffering and financial cost.[1,2] An important feature of dementia is the long preclinical phase, during which subtle cognitive deficits develop that can only be measured using dedicated neuropsychological tests.[3] The underlying etiology of dementia and cognitive decline is multi-factorial and involves different pathologies which interact and accumulate over the course of years.[4] In addition to beta-amyloid and tau pathology, the role of vascular pathology in the etiology of dementia and Alzheimer disease is increasingly being recognized.[5,6]

Atherosclerosis is highly frequent in the aging population and is considered the most important hallmark of vascular pathology.[7] Thus far, most studies have focused on atherosclerosis in the carotid bifurcation in relation to dementia.[8-10] Indeed, both carotid intima-media thickness and carotid plaques have been associated with dementia, including Alzheimer disease.[8-10] However, several important questions remain unanswered. First, atherosclerosis is a systemic disease, but its burden differs considerably across vessel beds.[7,11,12] It is therefore conceivable that the contribution of atherosclerosis to dementia may vary depending on the vessel bed. Such differential contribution of atherosclerosis in various vessel beds to disease risk has already been demonstrated for stroke, and even for mortality.[13,14] Second, the study of vascular factors in dementia is often complicated by stroke, which can act as intermediate factor.[9,10] It is therefore important to also investigate the role of atherosclerosis in dementia independent from stroke. Finally, given that both atherosclerosis and dementia develop over the course of years, it is important to study how atherosclerosis affects the preclinical phase of dementia, namely the period of cognitive decline without overt clinical disease.

Disentangling the exact role of atherosclerosis in dementia is important, because this knowledge may then serve as basis to develop opportunities for therapeutic or preventive intervention. Against this background, we aimed to study the relationship of atherosclerosis in the coronary arteries, aortic arch, extracranial and intracranial internal carotid arteries with incident dementia, including Alzheimer disease and the potential influence of stroke on these associations. Finally, we focused on the relationship of atherosclerosis with cognitive decline.

Methods

Setting

This study is based on the Rotterdam Study, a prospective, population-based study aimed at investigating determinants of chronic diseases in the elderly.[15] The original cohort comprised 7,983 participants aged 55 years or older and was extended in 2000-2001 with 3,011 persons.

At study entry and every 3 to 4 years, all participants are re-examined in a dedicated research center.

Between 2003 and 2006, all participants visiting the research center were invited to undergo non-enhanced computed tomography (CT). Therefore for the current study, 2003-2006 is taken as baseline. In total, we scanned 2,524 participants (response rate 78%). Both in 2003-2006 and the following visit in 2008-2012 persons underwent cognitive testing. The cohort was screened for dementia at baseline in order to exclude persons with prevalent dementia. From then onwards, the dementia-free cohort was followed-up for dementia through in-person screening at the follow-up visit and through continuous monitoring for dementia via computerized linkage between the study database and medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care, from baseline until April 27, 2012.This study was approved by the Medical Ethics Committee at Erasmus Medical Center, the Netherlands. All participants gave informed consent.

Sample for analysis

Figure 1 shows the composition of the study population. Due to the presence of a pacemaker, coronary stent implantations or image artefacts, 111 examinations from the 2,524 were not gradable, leaving a total of 2,413 participants with a complete CT examination. From these, 2,364 participants were at risk for developing dementia (incorrect dementia-screening or prevalent dementia excluded), encompassing the study population at baseline.

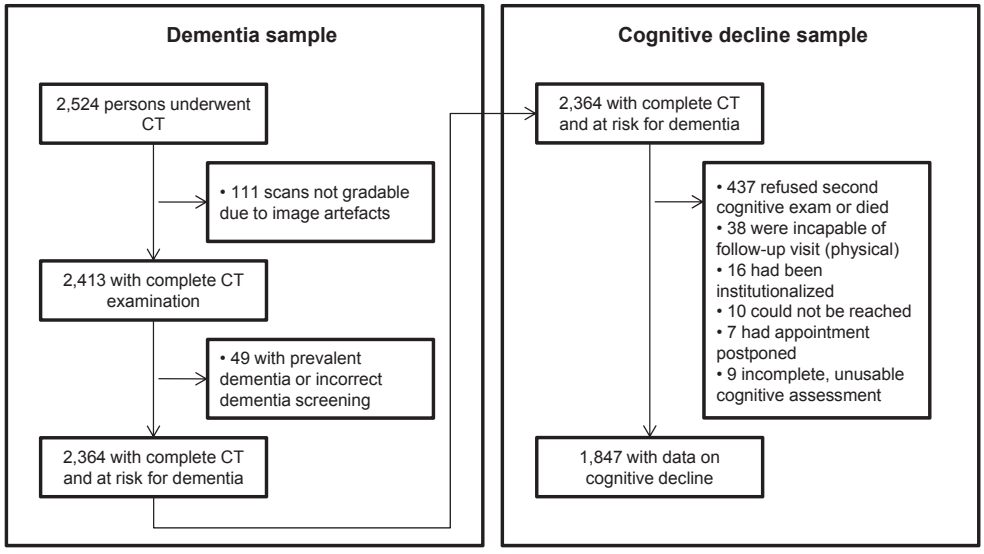

Figure 1. Flowchart of the composition of the study sample

From these 2,364 participants, 437 refused a second cognitive examination or had died during follow-up, 38 were incapable of follow-up visit (e.g. physical limitations), 16 had been institutionalized or moved, 10 could not be reached, for 7 participants the appointment was postponed for logistical reasons and in 9 participants the cognitive assessment was incomplete and could not be used. This left 1,847 participants with data on cognitive change (MMSE or at least one cognitive test).

CT acquisition and processing

We used a 16-slice (n=785) or 64-slice (n=1,739) multidetector computed tomography (CT) scanner (Somatom Sensation 16 or 64, Siemens, Forchheim, Germany) to perform non-contrast CT-scanning. Using a cardiac scan and a scan that reached from the aortic arch to the intracranial vasculature (1 cm above the sella turcica), we scanned the following vessel beds: the coronary arteries, the aortic arch, the extracranial carotid arteries, and the intracranial carotid arteries (Figure 2). Detailed information regarding imaging parameters of both scans is described elsewhere.[12]

We used dedicated commercially available software (Syngo CalciumScoring, Siemens, Germany) to quantify calcification volume in the coronary arteries, aortic arch, and extracranial carotid arteries.[12] For calcification in the intracranial carotid arteries we used a semi-automated scoring method which is described in detail elsewhere.[16] Briefly, we delineated calcification in the intracranial carotid arteries manually in every CT slice. Next, we calculated the volume of intracranial carotid artery calcification by multiplying the number of pixels above the threshold of 130 Hounsfield units[17] with the pixel size and slice increment.

The interrater reliability of this method is very good (intraclass correlation coefficient, 0.99).[16] Calcification volumes in each vessel bed are expressed in cubic millimeters. Correlations between calcification across the four vessel beds ranged from 0.5 to 0.6.[12,18]

Ascertainment of dementia

We screened participants for dementia at baseline and follow-up using a three-step protocol.[19,20] The first screening step consisted of the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. If participants were screen-positive (MMSE < 26 or GMS organic level > 0), they entered the second step which consisted of the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[20] Additionally, persons underwent history-taking, assessment of activities of daily living, informant interview, retrieval of relevant medical records, and additional neuropsychological testing. When information on neuroimaging was available (38/90 dementia cases; 42%), it was used for decision making on the diagnosis. If sufficient information was already obtained through the medical records, neuropsychological testing was not performed. Third, all remaining cases

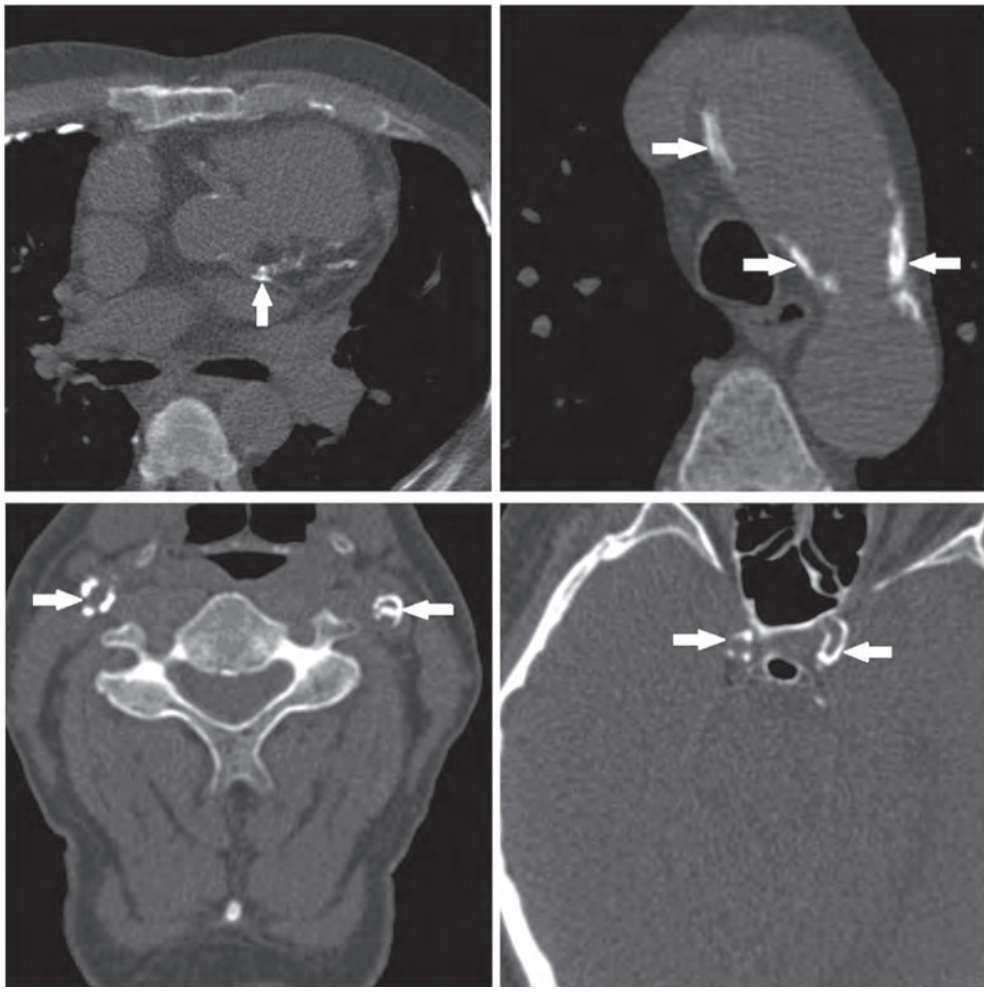

Figure 2. Examples of calcification in the four examined vessel beds
Arrows indicate atherosclerotic calcified lesions in: the left coronary artery (upper left), the aortic arch (upper right), the left and the right extracranial internal carotid arteries (lower left), and the left and right intracranial internal carotid arteries (lower right).

were discussed in a consensus panel consisting of research physicians led by an experienced neurologist, deciding on the final diagnosis in accordance with standard criteria using the DSM-III-R criteria for dementia. If applicable, subtyping of dementia was done using the NINCDS-ADRDA for Alzheimer disease and NINDS-AIREN for vascular dementia.[21,22] Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. This was important to identify

those persons that became demented, who did not visit our research center and therefore were not detected by our dementia screening. If available, the consensus panel used results from screening as well as information obtained by monitoring medical records to assess a dementia diagnosis. Consequently, in certain cases (18 out of 90 cases) information from both sources was used. For the remaining cases 53 were detected trough computerized linkage and 19 through return visits.

Assessment of cognitive function and cognitive decline

Both at baseline and at a follow-up examination in 2008-2012, participants underwent MMSE.[23] In addition, each participant underwent a more extensive neuropsychological test battery which consisted of the following tests: the Stroop test (reading, colour-naming, and interference subtask), the Letter-Digit Substitution Task (LDST), a Word-Fluency test (WFT) and a 15-Word verbal Learning Task (15-WLT).[24,25] We calculated decline for MMSE and each cognitive test by subtracting the test-score at follow-up from the test-score at baseline for each individual. The differences for cognitive tests were subsequently standardized (i.e. Z-scores) to aid comparisons across tests. Z-scores for each subtask of the Stroop test were inverted, because higher scores for these indicate worse performance whilst higher scores on the other tests indicate better performance. Finally, we averaged the Z-scores to yield a measure of global cognition.

Other measurements in the Rotterdam Study

We collected detailed information on cardiovascular risk factors by interview, physical examination and blood sampling.[15] The following cardiovascular risk factors were measured: obesity, hypertension, diabetes, hypercholesterolemia, low HDL-cholesterol level and smoking status. Obesity was defined as a body-mass index (BMI) ≥ 30 kg/m^2. Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of blood pressure lowering medication.[26] Diabetes was defined as fasting serum glucose levels ≥ 7.0 mmol/l and/or the use of anti-diabetic therapy.[27] Hypercholesterolemia was defined as total cholesterol concentration ≥ 6.2 mmol/l and/or the use of lipid lowering medication.[28] We defined low high-density lipoprotein (HDL)-cholesterol as HDL-cholesterol < 1.0 mmol/l.[28] Smoking was categorized into never or ever smoked. Level of education was assessed by self-report.[25] We performed *APOE*-genotyping on coded genomic DNA samples and coded it positive (carrier of one or two ε4-alleles) or negative (non-carrier). The definition of stroke was based on WHO criteria as a syndrome of rapidly developing symptoms of cerebral dysfunction lasting 24 hours or longer or leading to death, with apparent vascular cause.[29,30] History of stroke was assessed during the baseline interview and verified by reviewing medical records.[29]After enrolment, we continuously monitored participants for incident stroke through linkage of the study database with files from general practitioners. We also

checked nursing home physicians' files and files from general practitioners of participants who moved out of the district. From the 152 stroke-cases in our study, neuroimaging was available in 101 persons (66 %). When neuroimaging was not available, strokes were categorized as 'unspecified', but still considered as an event. Potential strokes were reviewed by research physicians and verified by an experienced stroke neurologist.[29]

Statistical analysis

Since calcification volume had a skewed, non-normal distribution, we used natural log-transformed values and added 1.0 mm^3 to the non-transformed values in order to deal with calcium scores of zero [Ln(calcification + 1.0 mm^3)].
We assessed the relationship between calcification volume in each vessel bed and the risk of dementia using Cox regression models. In the first model we adjusted for age, sex and educational level (model I). In a second model, we adjusted for cardiovascular risk factors (obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol levels and smoking status) and *APOE* ε4-carriership status (model II). Next, associations between calcification and dementia were reanalysed after exclusion of participants with prevalent stroke at baseline (n=78) or incident stroke during the dementia follow-up (n=74).
We used linear regression to study the relationship of calcification volume with decline in MMSE, global cognition and the standardized separate neurocognitive tests. The adjustments we performed were identical to those in models I and II used for dementia, with the addition of time interval between baseline and follow-up as covariate. Subsequent analyses were performed after exclusion of persons with stroke (n=90) and dementia (n=42).
IBM SPSS Statistics version 20 (International Business Machines Corporation, Armonk, New York) was used for statistical analyses.

Results

The baseline characteristics of our study participants are summarised in Table 1 (Supplementary table 1 also depicts the characteristics of the source population, from which the current sample was derived). The mean age at baseline was 69.4 ± 6.7 years and 52.3% of the participants were female. During 13,397 person years of follow-up, 90 participants developed dementia (incidence rate of 6.7 per 1000 person years). Of these, 73 were diagnosed with Alzheimer disease, 3 with vascular dementia and 14 with other/undetermined types of dementia. In total, 152 participants suffered a stroke of whom 13 later developed dementia. Hence, 77 participants developed dementia without previous stroke (63 with Alzheimer disease). On average, people declined 0.27 ± 2.00 points on the MMSE during a mean follow-up period of 6.0 ± 0.5 years.

Table 1. Baseline characteristics of the study population

Study population	N=2,364
Women	52.3%
Age, years	69.4 (6.7)
Highest education attained:	
Primary education	10.3%
Low level vocational training	20.8%
Medium level secondary training	17.9%
Medium level vocational to university training	49.1%
Obesity	23.9%
BMI, kg/m^2	27.7 (3.9)
Hypertension	73.7%
Systolic blood pressure, mmHg	146.7 (20.1)
Diastolic blood pressure, mmHg	80.3 (10.7)
Diabetes	11.2%
Serum glucose, mmol/L	5.7 (1.3)
Hypercholesterolemia	48.6%
Serum total cholesterol, mmol/L	5.7 (1.0)
Low HDL-cholesterol	10.6%
Serum HDL-cholesterol, mmol/L	1.4 (0.4)
Smoking, ever	67.5%
APOE ε4-carriers	25.6%

Values are means (standard deviations) for continuous variables and percentages for dichotomous variables.

We found that larger calcification volumes in the aortic arch, extracranial carotid arteries and intracranial carotid arteries, but not in the coronary arteries, were related to a higher risk of dementia (Table 2, model I). Additional adjustment for cardiovascular risk factors and *APOE* ε4-status did slightly attenuate these associations (Table 2, model II). We found similar associations for Alzheimer disease (Table 2).

After censoring for stroke, both extracranial and intracranial carotid artery calcification remained statistically significantly associated with dementia (hazard ratio (HR) per standard deviation (SD) increase in calcification volume: 1.32, 95% CI 1.02;1.71) and 1.34, 95% CI 1.01;1.78), respectively). Effect sizes for the remaining associations also remained similar, though statistically non-significant (Table 3).

For cognitive decline, we found that calcification in all vessel beds, including the coronary arteries, was associated with decline in global cognition and, apart from extracranial carotid artery calcification, with decline in MMSE (Table 4, model I). Additional adjustment for cardiovascular risk factors and *APOE* ε4 status attenuated most associations, but the associations

Table 2. Atherosclerotic calcification and the risk of dementia and Alzheimer disease

	Dementia		Alzheimer disease	
	n/N = 90/2364 HR (95% CI)	*P*	n/N = 73/2364 HR (95% CI)	*P*
Per SD increase in:		Model I		
Coronary calcification	1.10 (0.86;1.41)	0.44	1.16 (0.88;1.53)	0.31
Aortic arch calcification	1.38 (1.02;1.86)	0.04	1.46 (1.04;2.06)	0.03
Extracranial carotid calcification	1.39 (1.09;1.77)	<0.01	1.31 (1.01;1.72)	0.05
Intracranial carotid calcification	1.31 (1.01;1.70)	0.05	1.29 (0.96;1.74)	0.09
		Model II		
Coronary calcification	1.12 (0.85;1.48)	0.43	1.17 (0.85;1.60)	0.34
Aortic arch calcification	1.27 (0.93;1.73)	0.13	1.32 (0.93;1.89)	0.12
Extracranial carotid calcification	1.37 (1.05;1.79)	0.02	1.26 (0.94;1.68)	0.13
Intracranial carotid calcification	1.32 (0.98;1.77)	0.07	1.22 (0.88;1.70)	0.23

Model I: adjusted for age, sex, and level of education.
Model II: additionally adjusted for obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol levels, smoking status and *APOE* ε4-carrier status.
Abbreviations: n=number of cases; N=number of persons at risk; HR=hazard ratio; CI=confidence interval; SD=standard deviation.

Table 3. Atherosclerotic calcification and the risk of dementia and Alzheimer disease, censored for stroke

	Dementia		Alzheimer disease	
	n/N 77/2212 HR (95% CI)	*P*	n/N 63/2212 HR (95% CI)	*P*
Per SD increase in:				
Coronary calcification	1.05 (0.80;1.36)	0.74	1.09 (0.81;1.46)	0.57
Aortic arch calcification	1.34 (0.97;1.83)	0.07	1.32 (0.92;1.88)	0.13
Extracranial carotid calcification	1.32 (1.02;1.71)	0.04	1.24 (0.94;1.65)	0.13
Intracranial carotid calcification	1.34 (1.01;1.78)	0.05	1.28 (0.93;1.76)	0.12

Adjusted for age, sex, and level of education.
Abbreviations: n=number of cases; N=number of persons at risk; HR=hazard ratio; CI=confidence interval; SD=standard deviation.

of coronary artery calcification with MMSE and the association of intracranial carotid artery calcification with MMSE and global cognition remained statistically significant [MMSE per SD increase - coronary artery calcification <beta>= -0.15 (95% CI -0.25;-0.05); intracranial carotid artery calcification <beta> = -0.12 (95% CI -0.22;-0.01)] [global cognition per SD increase: intracranial carotid artery calcification <beta> = -0.03 (95% CI -0.06;0.00)] (Table 4, model 2). After censoring for stroke, coronary artery calcification remained statistically

Table 4. Calcification in different vessel beds and global cognitive decline

	Global cognition		MMSE	
	Difference in Z-score (95% CI)	*P*	Difference in points (95% CI)	*P*
Per SD increase in:		Model I		
Coronary calcification	-0.03 (-0.06;0.00)	0.03	-0.15 (-0.25;-0.05)	<0.01
Aortic arch calcification	-0.03 (-0.05;0.00)	0.06	-0.11 (-0.21;-0.01)	0.03
Extracranial carotid calcification	-0.03 (-0.06;0.00)	0.04	-0.07 (-0.16;0.03)	0.19
Intracranial carotid calcification	-0.04 (-0.07;-0.02)	<0.01	-0.09 (-0.19;0.00)	0.06
		Model II		
Coronary calcification	-0.02 (-0.05;0.01)	0.13	-0.16 (-0.27;-0.05)	<0.01
Aortic arch calcification	-0.01 (-0.04;0.02)	0.39	-0.11 (-0.22;-0.01)	0.03
Extracranial carotid calcification	-0.02 (-0.05;0.01)	0.22	-0.07 (-0.17;0.04)	0.22
Intracranial carotid calcification	-0.03 (-0.06;0.00)	0.03	-0.12 (-0.22;-0.01)	0.03

Model I: adjusted for age, sex, time interval and level of education.
Model II: additionally adjusted for obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol levels, smoking status and *APOE* ε4-carrier status
Abbreviations: CI=confidence interval; SD=standard deviation.

significantly associated with MMSE (MMSE per SD increase: coronary artery calcification <beta>= -0.11 (95% CI 0.21;-0.01)]. Effect sizes for the remaining associations were no longer statistically significant (Supplementary table 2). After we excluded all persons who developed dementia during follow-up, we still found similar associations of calcification with cognitive decline (Supplementary table 2).
Associations of calcification with the separate cognitive tests are shown in Table 5 (A and B). We found most prominent associations of calcification volume in the coronary arteries and the intracranial carotid arteries with decline in scores on the LDST.

Discussion

In this sample of community-dwelling middle-aged and elderly persons, we found that atherosclerotic calcification, in particular in the extracranial carotid arteries, was related to a higher risk of dementia, including Alzheimer disease. Furthermore, we found atherosclerotic calcification to be associated with cognitive decline in non-demented persons.
Strengths of our study include the longitudinal population-based setting, the image-based quantification of atherosclerosis, and the focus on both cognitive decline and dementia.

Table 5A. Calcification in different vessel beds and cognitive decline per single test (Stroop Test and LDST)

	Stroop Reading*		Stroop Naming*		Stroop CWI*		LDST	
	Difference in Z-score (95% CI)	*P*	Difference in Z-score (95% CI)	*P*	Difference in Z-score (95% CI)	*P*	Difference in Z-score (95% CI)	*P*
Per SD increase in:				Model I				
Coronary calcification	0.01(-0.05;0.07)	0.72	0.02(-0.03;0.08)	0.43	-0.01(-0.06;0.05)	0.79	-0.06(-0.12;-0.01)	0.02
Aortic arch calcification	-0.02(-0.08;0.03)	0.38	-0.00(-0.05;0.05)	0.97	-0.01(-0.07;0.04)	0.65	-0.06(-0.11;0.00)	0.04
Extracranial carotid calcification	-0.01(-0.06;0.05)	0.79	-0.02(-0.07;0.03)	0.47	0.01(-0.04;0.07)	0.67	-0.06(-0.11;-0.01)	0.02
Intracranial carotid calcification	-0.02(-0.07;0.04)	0.57	-0.02(-0.07;0.04)	0.54	-0.02(-0.07;0.03)	0.42	-0.05(-0.10;0.00)	0.03
				Model II				
Coronary calcification	0.02(-0.04;0.08)	0.49	0.03(-0.03;0.08)	0.39	0.01(-0.05;0.07)	0.74	-0.06(-0.12;-0.01)	0.03
Aortic arch calcification	-0.02(-0.07;0.04)	0.60	0.00(-0.06;0.05)	0.94	0.01(-0.05;0.06)	0.78	-0.05(-0.10;0.01)	0.08
Extracranial carotid calcification	0.02(-0.04;0.08)	0.57	-0.01(-0.06;0.05)	0.84	0.04(-0.02;0.09)	0.24	-0.05(-0.10;0.01)	0.10
Intracranial carotid calcification	0.00(-0.05;0.06)	0.88	-0.02(-0.07;0.04)	0.53	-0.02(-0.07;0.04)	0.58	-0.06(-0.11;-0.01)	0.03

Model I: adjusted for age, sex, time interval and level of education.

Model II: additionally adjusted for obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol levels, smoking status and *APOE* ε4-carrier status.

*Inverted scores; lower scores indicate worse performance.

Abbreviations: CI=confidence interval; SD=standard deviation; CWI=Colour Word Interference; LDST=Letter–Digit Substitution Task.

Table 5B. Calcification in different vessel beds and cognitive decline per single test (WFT and 15-WLT)

	WFT		15-WLT DR		15-WLT IR	
	Difference in Z-score (95% CI)	*P*	Difference in Z-score (95% CI)	*P*	Difference in Z-score (95% CI)	*P*
Per SD increase in:			Model I			
Coronary calcification	-0.03(-0.08;0.03)	0.31	-0.04(-0.09;0.02)	0.24	-0.04(-0.10;0.02)	0.20
Aortic arch calcification	-0.01(-0.06;0.04)	0.68	-0.02(-0.08;0.04)	0.48	-0.03(-0.08;0.03)	0.38
Extracranial carotid calcification	0.00(-0.05;0.05)	0.88	-0.07(-0.13;-0.02)	0.01	-0.01(-0.06;0.05)	0.76
Intracranial carotid calcification	-0.06(-0.11;-0.01)	0.03	-0.04(-0.09;0.02)	0.18	-0.02(-0.08;0.03)	0.44
			Model II			
Coronary calcification	-0.03(-0.08;0.03)	0.36	-0.03(-0.09;0.03)	0.34	-0.04(-0.10;0.02)	0.20
Aortic arch calcification	0.00(-0.05;0.06)	0.93	-0.01(-0.07;0.05)	0.85	-0.02(-0.08;0.04)	0.48
Extracranial carotid calcification	0.02(-0.04;0.07)	0.55	-0.08(-0.14;-0.02)	0.01	-0.02(-0.08;0.04)	0.49
Intracranial carotid calcification	-0.04(-0.10;0.01)	0.10	-0.02(-0.08;0.04)	0.47	-0.02(-0.08;0.04)	0.44

Model I: adjusted for age, sex, time interval, and level of education.

Model II: additionally adjusted for obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol levels, smoking status and *APOE* ε4-carrier status.

Abbreviations: CI=confidence interval; SD=standard deviation; WFT=Word Fluency Task; WLT DR=15-word learning test delayed recall; WLT IR=15-word verbal learning test immediate recall.

Moreover, our close collaborations with general practitioners in the study area, in combination with the structure of the Dutch health care system allowed us to accomplish virtually complete follow-up with regard to development of dementia. The incidence rate of dementia in our sample was comparable to incidence rates reported in other studies.[20,31]

Several potential limitations should also be addressed. First, additional adjustment for covariates affected the results regarding dementia and cognitive decline. Yet, in most cases the effect estimates remained largely unchanged. It is possible that addition of multiple covariates to the model in combination with the relatively small sample size may have led to less statistical power. Importantly, this does not necessarily imply that there is no real association between atherosclerosis and the outcomes. Second, we performed multiple statistical tests which might have inflated our type I error. However, an important consideration here is that most tests are actually not completely independent (e.g. the various cognitive domains are correlated and calcification in the different vessels is correlated). Therefore, conventional corrections for multiple testing would be overconservative.[32] Another consideration is that calcification is only a part of the atherosclerotic plaque. Using non-enhanced CT it is not possible to visualize the complete atherosclerotic plaque area and thus potentially interesting information on additional plaque characteristics, such as shape, stenosis or ulceration could not be measured. Nonetheless, strong evidence suggests that CT-based calcification volume provides an adequate reflection of the total underlying plaque burden.[33] For the assessment of calcification we used two types of multidetector CT-scanners (16 slice and 64 slice CT), which could have influenced the measurements. However, post-hoc analyses with adjustment for scanner type did not change the results. Next, due to selective participation of younger and healthier subjects, our results may be underestimating true associations. We also note that structural neuroimaging was not available in all participants to aid in the diagnostic process. This could have led to potential misclassification of vascular dementia as AD. Yet, we expect this misclassification to be minimal, given that we always used all clinical information. Finally, we did not have neuropathological confirmation of dementia.

We found that atherosclerotic calcification in multiple vessel beds was related to a higher risk of dementia. Several explanations may underlie this association. First, our findings could be explained by an intermediary role for stroke. Atherosclerosis is a powerful risk factor for stroke[34], and in turn stroke-patients have an almost two-fold increased risk of dementia.[6,35] However, censoring at time of stroke changed little in our associations of calcification and incident dementia. A similar absence of effect of stroke has been shown for the relationship between carotid intima-media thickness and dementia.[9,10] Another point of consideration is that atherosclerosis in the carotid artery represents the strongest location of atherosclerosis related to stroke.[36,37] In contrast, we found associations with dementia for other vessel beds as well. This also points towards our findings being independent from stroke. Actually, this sug-

gests that generalized atherosclerosis, which probably is a better reflection of one's vascular status rather than localized atherosclerosis, associates with dementia.

A second explanation linking atherosclerosis to dementia is subclinical small vessel disease.[6,38] Autopsy studies provide strong evidence that the majority of patients with Alzheimer disease show small vessel disease in their brain.[6] This includes infarcts, micro-infarcts, microbleeds, demyelinization and axonal damage.[6] In-vivo, imaging studies have shown that MRI-markers of small vessel disease, e.g. white matter lesions and lacunar infarcts, are associated with the risk of dementia.[6,39,40] We have previously demonstrated that atherosclerotic calcification in all four vessel beds is strongly associated with these MRI-markers of subclinical vascular brain disease.[18] Together, this points towards a role for small vessel disease in the association of atherosclerosis with dementia.

Finally, chronic hypoperfusion may explain the association between atherosclerosis and dementia, especially Alzheimer disease.[6] As a consequence of slowly-progressing structural changes of cerebral vessels due to atherosclerosis, cerebral perfusion is impaired which could lead to subclinical vascular brain disease on the one hand, but may also cause loss of functionality of the blood-brain barrier. This in turn might allow increased parenchymal deposition of beta-amyloid protein and/or impaired amyloid clearance and thereby the formation of amyloid plaques, an important characteristic of Alzheimer disease.[5,6,41]

Research on dementia is very often challenging due to the possibility of competing risks.[9] In our study competing risk due to mortality might explain the lack of association between coronary artery calcification and dementia. The coronary arteries are the single most important location of atherosclerosis for risk of cardiac events, including cardiac death.[13] It is therefore conceivable that persons in our study with largest load of coronary calcification died of competing risks and therefore did not remain at risk to develop dementia. Indeed, post-hoc analyses revealed that especially coronary artery calcification was associated with mortality.

By investigating cognitive decline in addition to the endpoint of dementia, we managed to circumvent to a certain extent competing risks. We found that atherosclerotic calcification in all locations, including the coronary arteries, was associated with cognitive decline. Moreover, when we repeated our analyses after excluding persons who converted to dementia during follow-up, we found that the results remained unchanged. This implies that our results were unlikely to be driven by preclinical dementia, but that atherosclerosis already plays an important role in the early stages of cognitive deterioration, presumably already years before the possible conversion to clinical dementia. Whereas our study had a follow-up period of 6 years, others found carotid atherosclerosis to be associated with cognitive decline over a 10-year period.[42,43] These findings indicate a potential window of opportunity for treatment of atherosclerosis which could possibly delay or even stop the cognitive decline and ultimately aid in the prevention of dementia.

When investigating the cognitive tests separately, in general, we found small effects of calcifi-

cation on the performance on the tests. In agreement with others[44], we found strong associations between atherosclerosis and the LDST, which primarily assesses processing speed. Yet, it was surprising that we did not find strong associations between atherosclerosis and executive function.[42,44] This might be because our assessment of executive function was not detailed or sensitive enough to assess small changes. In our test battery there was namely only one test that primarily investigated executive function; the WFT. We only found an association of intracranial carotid artery calcification with this test.

In conclusion, our findings further establish the role of atherosclerosis in the etiology of dementia and cognitive decline. This calls for studies evaluating whether interventions targeted at reducing or stabilizing atherosclerosis would have a beneficial effect on the occurrence of dementia.

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Supplementary table 1. Vascular risk profiles across participants in the current study samples and the total Rotterdam Study cohort

	Rotterdam Study participants in present analyses		Other Rotterdam Study participants*
	N = 2,364	N = 1,847	N = 3,686
Women	52.3%	52.9%	63.1%
Age, years	69.4 (6.7)	68.4 (5.9)	75.3 (7.6)
Obesity	23.9%	23.8%	18.3%
BMI, kg/m^2	27.7 (3.9)	27.7 (3.9)	27.5 (4.3)
Hypertension	73.7%	72.3%	79.0%
Systolic blood pressure, mmHg	146.7 (20.1)	145.6 (19.1)	152.1 (22.1)
Diastolic blood pressure, mmHg	80.3 (10.7)	80.1 (10.4)	79.3 (11.2)
Diabetes	11.2%	10.1%	14.9%
Serum glucose, mmol/L	5.7 (1.3)	5.7 (1.2)	6.0 (1.6)
Hypercholesterolemia	48.6%	49.1%	40.0%
Serum total cholesterol, mmol/L	5.7 (1.0)	5.7 (1.0)	5.6 (1.0)
Low HDL-cholesterol	10.6%	10.0%	7.4%
Serum HDL-cholesterol, mmol/L	1.4 (0.4)	1.4 (0.4)	1.5 (0.4)
Smoking, ever	67.5%	66.9%	65.2%
APOE ε4-carriers	25.6%	25.1%	25.6%

* These are all other Rotterdam Study-participants that participated in the follow-up visit [n = 6,050 (all) – 2,364 (current study) = 3,686].
Values are means (standard deviations) for continuous variables and percentages for dichotomous variables.

Supplementary table 2. Calcification in different vessel beds and cognitive decline after excluding persons who suffered a stroke (upper panel) or developed dementia (lower panel)

	Global cognition		**MMSE**	
	Difference in Z-score (95% CI)	*P*	Difference in points (95% CI)	*P*
Per SD increase in:	**Stroke excluded (n = 90)**			
Coronary calcification	-0.03 (-0.05;0.00)	0.06	-0.11 (-0.21;-0.01)	0.03
Aortic arch calcification	-0.02 (-0.05;0.00)	0.10	-0.07 (-0.17;0.03)	0.15
Extracranial carotid calcification	-0.02 (-0.05;0.01)	0.13	-0.02 (-0.12;0.08)	0.69
Intracranial carotid calcification	-0.04 (-0.07;-0.02)	<0.01	-0.07 (-0.16;0.03)	0.19
	Dementia excluded (n = 42)			
Coronary calcification	-0.03 (-0.06;-0.01)	0.02	-0.13 (-0.22;-0.03)	<0.01
Aortic arch calcification	-0.02 (-0.05;0.00)	0.09	-0.09 (-0.18;0.00)	0.06
Extracranial carotid calcification	-0.03 (-0.05;0.00)	0.05	-0.03 (-0.12;0.06)	0.47
Intracranial carotid calcification	-0.04 (-0.06;-0.01)	<0.01	-0.09 (-0.18;0.00)	0.05

Adjusted for age, sex, time interval and level of education.
Abbreviations: CI=confidence interval; SD=standard deviation; n=number of cases.

Chapter 3.4

Cerebral vasomotor reactivity and the risk of mortality

Marileen L.P. Portegies*, Renée F.A.G. de Bruijn*,
Albert Hofman, Peter J. Koudstaal, M. Arfan Ikram

* These authors contributed equally to the manuscript

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Abstract

Background: Accumulating vascular pathology in cerebral arteries leads to impaired cerebral vasomotor reactivity. In turn, impaired cerebral vasomotor reactivity is a risk factor for stroke in clinical populations. It remains unclear whether impaired cerebral vasomotor reactivity also reflects more systemic vascular damage. We investigated whether cerebral vasomotor reactivity is associated with the risk of mortality, focusing particularly on cardiovascular mortality independent from stroke.

Methods: Between 1997-1999, 1,695 participants from the Rotterdam Study underwent cerebral vasomotor reactivity measurements using transcranial Doppler. Follow-up was complete until January 1, 2011. We assessed the associations between cerebral vasomotor reactivity and mortality using Cox proportional hazards models, adjusting for age, sex, and blood pressure changes and subsequently for cardiovascular risk factors. We additionally censored for incident stroke.

Results: During 17,004 person-years 557 participants died, of whom 181 due to a cardiovascular cause. In the fully adjusted model, the hazard ratio per SD decrease in vasomotor reactivity was 1.10 (95% CI 1.01;1.19) for all-cause mortality, 1.09 (95% CI 0.94;1.26) for cardiovascular mortality, and 1.10 (95% CI 0.99;1.21) for non-cardiovascular mortality. These associations remained unchanged after censoring for incident stroke.

Conclusions: We found that lower cerebral vasomotor reactivity is associated with an increased risk of death. Incident stroke does not affect this association, suggesting that a lower cerebral vasomotor reactivity reflects a generally impaired vascular system.

Introduction

Vascular diseases are the main cause of mortality worldwide and lead to considerable societal burden, both in terms of care and cost. The World Health Organization estimates that by 2030, more than 23 million people will die yearly from vascular diseases.[1] Despite the acute clinical presentation, an important feature of vascular diseases is the long preclinical phase, during which various pathologies interact leading to accumulating vascular damage. These pathologies include atherosclerosis, arterial stiffening, inflammation, and endothelial damage.[2-6] In the brain, this pathologic process ultimately manifests itself as either ischemic or hemorrhagic stroke.[7]

A cornerstone of preventive research has been to identify markers that reflect such preclinical vascular pathology and thus may predict shorter survival. For cerebrovascular damage, diminished vasomotor reactivity has been identified in recent years as a prognostic marker.[8] Cerebral vasomotor reactivity reflects the ability of the cerebral arterioles to dilate in the event of hypercapnia to improve cerebral blood flow.[9,10] Clinically, cerebral vasomotor reactivity can be measured using transcranial Doppler. Most studies investigating vasomotor reactivity were in clinical populations of patients with carotid artery stenosis. In these studies impaired vasomotor reactivity was associated with an increased risk of stroke and TIA.[8,11-16] However, its role in the general community-dwelling population is less clear. Vasomotor reactivity has been measured within the population-based Rotterdam Study, but no association between vasomotor reactivity and stroke was found.[17,,18]

Still, the question remains whether impaired cerebral vasomotor reactivity associates with poorer survival in a general elderly population. Specifically, it is unknown whether any such associations are driven by stroke, or whether cerebral vasomotor reactivity actually reflects more systemic vascular damage. Therefore, we investigated the association of cerebral vasomotor reactivity with all-cause mortality and cardiovascular mortality in a community-dwelling elderly population. Furthermore, we studied whether any associations were independent of incident stroke.

Materials and Methods

Setting

This study is part of the Rotterdam Study, a prospective population-based cohort study that started in 1990 among inhabitants of 55 years and older residing in Ommoord, a suburb of Rotterdam, the Netherlands. Of the 10,215 invited inhabitants, 7,983 agreed to participate in the baseline examinations. Up until 2013, there have been 4 follow-up examinations. Details of the study have been described elsewhere.[19] The medical ethics committee at Erasmus Uni-

versity of Rotterdam approved the study and written informed consent was obtained from all participants. For the current study, the second follow-up examination from 1997-1999 was used as baseline, because transcranial Doppler measurements were performed only at that visit.

Transcranial Doppler assessment

At the examination in 1997-1999, participants underwent transcranial Doppler ultrasonography (Multi-Dop X-4; DWL, Sipplingen, Germany). Vasomotor reactivity was measured as follows[17]: the cerebral blood flow velocity was measured at the middle cerebral artery continuously. End diastolic, peak systolic, and mean cerebral blood flow velocities were recorded automatically. Mean blood flow velocity was calculated automatically as (1/3 * (peak systolic flow velocity + 2 * end diastolic flow velocity)).[18] Blood pressure was measured automatically (Dynamap, Datascope, The Netherlands) before and during the transcranial Doppler recordings. The participants first breathed room air through an anesthetic mask, tightly fit over mouth and nose, until a steady expiratory end tidal CO_2 was obtained. One way valves were placed in the tubes for inspiration and expiration. End tidal CO_2 pressure (kPa), measured in the exhaled air, was recorded continuously with a CO_2 analyzer (Multinex; Datascope, Hoevelaken, the Netherlands). End expiratory CO_2 was assumed to reflect arterial CO_2.[20] Participants then inhaled a mixture of 5% carbon dioxide in 95% oxygen for 2 minutes. Vasomotor reactivity was defined as the percentage increase in mean cerebral blood flow velocity during inspiration of 5% CO_2, divided by the absolute increase in end tidal CO_2 in the same time period (%/kPa). TCD-8 DWL special software (VMR- CO_2) was used. All transcranial Doppler data were stored on hard disk for offline analysis.

Assessment of mortality

Deaths were continuously reported through automatic linkage of general practitioner files. In addition, municipal records were checked bimonthly for information on vital status. Information about cause and circumstances of death was obtained from general practitioner and hospital records. Research physicians reviewed all available information and coded the events according to the International Classification of Diseases, 10th edition (ICD-10). If the cause of death was coded as I20-I25, I46, I50, I61, I63, I64, I66, I68-70, or R96, the cause of death was labeled as cardiovascular. A consensus panel, led by a physician with expertise in cardiovascular disease, adjudicated the final cause of death according to the ICD-10 codes using standardized definitions, as described in detail previously.[21] The follow-up was complete until January 1, 2011, for 97.1% of potential person-years.

Assessment of stroke

At study entry, history of stroke was assessed using home interviews and confirmed by reviewing medical records. Once participants enter the Rotterdam Study, they are continuously followed-up for stroke through automatic linkage of general practitioner files with the study database. Also, nursing home physicians' files and files from general practitioners of participants that moved out of the district were checked on a regular basis. Of the potential strokes, additional hospital and general practitioner information was collected. Research physicians reviewed the stroke information and an experienced neurologist adjudicated the strokes using standardized definitions, as described in detail previously.[22] The follow-up was complete until January 1, 2011, for 97.3% of potential person-years.

Other measurements

Covariates were measured during the same examination round as transcranial Doppler measurements were performed (1997-1999). Smoking status and medication use were assessed using a home interview. Smoking was classified into current smoking, former smoking, or never smoking. Diabetes mellitus was defined as having a fasting glucose level of 7.0 mmol/L or higher or using blood glucose-lowering medication. Total cholesterol and HDL-cholesterol levels were acquired by an automated enzymatic procedure. Blood pressure was measured at the research center twice in the sitting position on the right arm with a random-zero sphygmomanometer. The average of the two measurements was used in the analyses. Blood pressure was also measured prior to and during the vasomotor reactivity measurements. We used the difference between these two measurements in the analyses since changes in blood pressure caused by CO_2 inhalation can influence vasomotor reactivity measurements.[23] Prevalent vascular disease (myocardial infarction, CABG, PCI, heart failure, and peripheral arterial disease) was, except for peripheral arterial disease, assessed through active follow-up and adjudicated using standardized definitions, as described in detail previously.[21] Peripheral arterial disease was assessed using the ankle-brachial index. Ankle-brachial index was assessed by computing the ratio of systolic blood pressure at the right and left ankle to the systolic blood pressure at the right arm. The lowest value was used in the analyses. Values of ankle-brachial index greater than 1.4 were excluded because high ankle-brachial index might represent a different underlying pathology. Peripheral arterial disease was defined as an ankle-brachial index of 0.9 or less.[24] To measure carotid intima-media thickness (cIMT), ultrasonography of the left and right carotid arteries was performed with a 7.5-MHz linear array transducer (ATL UltraMark IV; Advanced Technology Laboratories, Bethel, Washington). The maximal cIMT, summarized as the mean of the maximal measurements from the near and far walls of both the left and right sides, was used for analysis.[24,25]

Study population

Of the 5,990 participants that were alive in 1997-1999, 4,797 people participated in the examination used as baseline for this study. Of these, 4,215 visited the study center. Due to lack of technical support, vasomotor reactivity measurements started later in the examination round (from July 1, 1997) and could only be offered to 2,732 random participants. After excluding participants with prevalent stroke at time of transcranial Doppler assessment (n=100), 2,632 participants were eligible for transcranial Doppler assessment. Of these, 937 participants were excluded because of window failure on both sides (n=656), restlessness, anxiety, and discomfort (n=56), or missing data for other reasons (n=225). This left 1,695 participants eligible for the analysis of this study. People with a prevalent stroke (i.e. stroke before vasomotor reactivity measurement) have a higher probability of vascular damage of the middle cerebral artery where vasomotor reactivity was measured. Moreover, these people are both at a higher risk of a recurrent stroke and at a higher risk of mortality compared to people without prevalent stroke. Including such people with prevalent stroke into our analysis, even if they had a remote stroke, would therefore bias our results.

Statistical analyses

We investigated the associations of vasomotor reactivity with all-cause mortality, cardiovascular mortality, non-cardiovascular mortality, and stroke using Cox proportional hazards models. We used these models since we investigated time-to-event data. The underlying time-scale in these models was the follow-up time in years, which was complete until January 1, 2011. Participants were censored within this follow-up period at date of death, date of loss to follow-up, or January 1, 2011, whichever date came first.

Because of a right skewed distribution of vasomotor reactivity, we first performed a natural logarithmic transformation to obtain a roughly normal distribution of the data. Logarithmic transformed vasomotor reactivity was entered continuously per standard deviation (SD) decrease into the models, because a decrease reflects an impaired reactivity. We presented the results per standard deviation merely for a uniform representation of the data, this presentation was also used in the previous paper of Bos MJ et al.[18] Furthermore, we studied vasomotor reactivity in quartiles taking the upper quartile as reference. All models were adjusted for age, sex, and blood pressure changes during vasomotor reactivity measurement. We adjusted subsequently for current smoking, former smoking, use of blood pressure lowering medication, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, HDL-cholesterol, use of statins, history of vascular disease, and carotid intima-media thickness for being potential confounders. Missing data on covariates (8.4% or less) were imputed based on sex and age using linear regression models.

To investigate whether vasomotor reactivity within normal ranges was associated with

mortality, we repeated the analysis after excluding participants with an exhausted vasomotor reactivity (lower than 5.3%/kPa, as described previously).[12]
To assess the role of stroke on these associations, we related vasomotor reactivity to stroke as well as to mortality after censoring for stroke.
We performed an additional analysis to investigate whether diminished vasomotor reactivity acts as intermediate between various cardiovascular risk factors and risk of mortality. We associated the various cardiovascular risk factors with mortality and investigated whether adjustment for vasomotor reactivity affected these associations.
All analyses were done using the IBM SPSS statistics version 20.0 (IBM Corp, Armonk, NY, USA).

Results

Table 1 shows baseline characteristics of the study population. Non-participants were older and more often women compared to participants. Also, they smoked less, had a higher HDL-cholesterol, a larger carotid intima-media thickness, had more often prevalent vascular disease, and used more often blood pressure lowering medication and statins than participants.
The average follow-up duration for total mortality was 10.0 years, during which a total of 557 of the 1,695 participants died, of whom 181 due to a cardiovascular cause and 376 due to a non-cardiovascular cause. The most important cardiovascular causes of death were stroke (N=41), heart failure (N=39), cardiac arrest (N=29), other sudden death with unknown cause (N=24), and acute myocardial infarction (N=26). The most important non-cardiovascular causes of death were cancer (N=170, especially lung (N=45), colon (N=22), pancreas (N=15), and breast cancer (N=10)), and dementia (N=40). Of the non-participants, 457 of the 1,037 participants (44.1%) died. This difference was statistically significant compared to the number of deaths in the study population.
A total of 168 participants suffered from a stroke of which 92 participants died, either due to the stroke or other causes. After censoring for stroke, a total of 465 participants died, 131 due to a cardiovascular cause and 334 due to a non-cardiovascular cause. The most important cardiovascular causes of death in this last group were heart failure (N=36), cardiac arrest (N=29), other sudden death with unknown cause (N=21), and acute myocardial infarction (N=24).
Table 2 shows the hazard ratios of all-cause mortality. A lower vasomotor reactivity was associated with a higher risk of all-cause mortality (hazard ratio (HR) per SD decrease in vasomotor reactivity 1.12, 95% confidence interval (CI) 1.03;1.21). These associations remained unchanged after additional adjustments (HR 1.10, 95% CI 1.01;1.19). Also, people in the

Table 1. Baseline characteristics

	Participants	Non-participants
	N=1,695	N=1,037
Age, mean (SD), years	70.7 (6.3)	73.2 (6.8)†
Follow-up time mortality, mean (SD), years	10.0 (3.0)	9.5 (3.3)†
Female, No. (%)	785 (46.3)	760 (73.3)†
Systolic blood pressure, mean (SD), mmHg	142.7 (20.8)	145.1 (21.4)
Diastolic blood pressure, mean (SD), mmHg	75.9 (11.1)	75.2 (11.0)
Blood pressure lowering medication, No. (%)	384 (23.2)	293 (29.2)†
Diabetes mellitus, No. (%)	158 (9.5)	111 (10.9)
Former smoking, No. (%)	990 (58.8)	447 (43.5)†
Current smoking, No. (%)	311 (18.5)	176 (17.1)
Total cholesterol, mean (SD), mmol/L	5.81 (0.99)	5.85 (1.01)
HDL-cholesterol, mean (SD), mmol/L	1.38 (0.38)	1.42 (0.39)†
Statins, No. (%)	216 (12.9)	154 (15.1)†
History of vascular disease, No. (%)	368 (23.3)	256 (27.1)†
Carotid intima-media thickness, mean (SD), mm	1.06 (0.18)	1.09 (0.20)†
Difference in systolic blood pressure before and during measurement, mean (SD), mmHg	14.9 (13.8)	NA
Difference in diastolic blood pressure before and during measurement, mean (SD), mmHg	5.7 (7.0)	NA
Vasomotor reactivity, median (IQR)*, %/kPa	39.3 (28.1 – 54.0)	NA

Data are presented as mean (standard deviations) or No. (%) unless otherwise specified. Percentages are calculated without missing data. For all reported variables, missing numbers occurred in 8.4% or less of all participants.

* Median (interquartile range) presented because of skewed distribution.

† Significantly different ($p<0.05$) between participants and non-participants, after sex and age adjustment – if applicable.

Abbreviations: N=number of participants; SD=standard deviation; HDL=high-density lipoprotein; NA=not available; IQR=interquartile range.

lowest two quartiles had an increased risk of death compared to the upper quartile (Table 2). Figure 1 shows the corresponding Kaplan-Meier curves for these associations.

Associations between vasomotor reactivity and mortality were stronger for cardiovascular mortality (HR per SD decrease 1.15, 95% CI 1.00;1.32), whilst the associations for non-cardiovascular mortality were weaker (HR 1.10, 95% CI 1.00;1.21) (Table 3). After additional adjustments, the association with cardiovascular mortality attenuated slightly and became statistically non-significant (HR 1.09, 95% CI 0.94;1.26). Again, people in the lowest two quartiles had an increased risk of death compared to the upper quartile (Table 2). However, we could not observe a clear dose-response relation between lower vasomotor reactivity and

Table 2. Vasomotor reactivity and the risk of all-cause mortality

Vasomotor reactivity*	n/N	Model I HR (95% CI)	Model II HR (95% CI)
Quartile 1	182/423	1.40 (1.10;1.80)	1.30 (1.01;1.67)
Quartile 2	154/424	1.36 (1.06;1.74)	1.33 (1.03;1.71)
Quartile 3	117/424	1.04 (0.79;1.35)	1.07 (0.82;1.40)
Quartile 4	104/424	1 (reference)	1 (reference)
Per SD decrease	*557/1,695*	*1.12 (1.03;1.21)*	*1.10 (1.01;1.19)*

Values are hazard ratios with 95% confidence intervals.

The range of vasomotor reactivity for each quartile was as follows: quartile 1: 0–28.1 %/kPa, quartile 2: 28.1-39.3 %/kPa, quartile 3: 39.3-54.0 %/kPa, quartile 4: 54.0-229.3 %/kPa.

* Vasomotor reactivity was natural log transformed.

Model I: adjusted for age, sex and difference in blood pressure before and during vasomotor reactivity measurement.

Model II: adjusted for age, sex, current smoking, former smoking, use of blood pressure lowering medication, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, HDL-cholesterol, use of statins, history of vascular disease, carotid intima-media thickness, and difference in blood pressure before and during vasomotor reactivity measurement.

Abbreviations: n=number of deaths; N=number of people at risk; HR=hazard ratio; CI=confidence interval; SD=standard deviation; HDL=high-density lipoprotein.

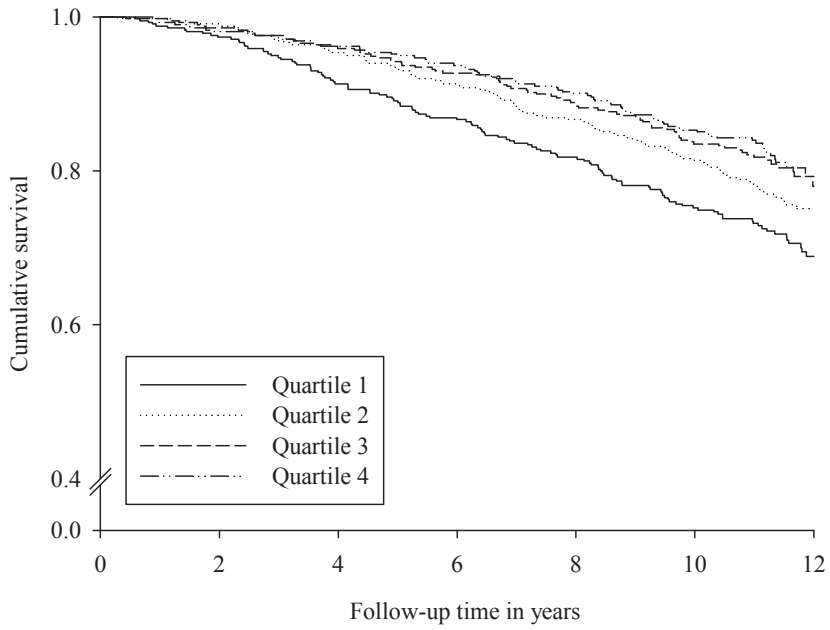

Figure 1. Kaplan-Meier curve for crude survival per quartile of vasomotor reactivity

Quartile 1 represents the lowest vasomotor reactivity, quartile 4 the highest. The range of vasomotor reactivity for each quartile was as follows: quartile 1: 0–28.1 %/kPa, quartile 2: 28.1-39.3 %/kPa, quartile 3: 39.3-54.0 %/kPa, quartile 4: 54.0-229.3 %/kPa.

Table 3. Vasomotor reactivity and the risk of cardiovascular and non-cardiovascular mortality

	Cardiovascular mortality			Non-cardiovascular mortality		
Vasomotor reactivity*	**n/N**	**Model I HR (95% CI)**	**Model II HR (95% CI)**	**n/N**	**Model I HR (95% CI)**	**Model II HR (95% CI)**
Quartile 1	56/423	1.62 (1.01;2.59)	1.36 (0.84;2.18)	126/423	1.33 (0.99;1.78)	1.28 (0.95;1.72)
Quartile 2	59/424	1.99 (1.26;3.15)	1.93 (1.21;3.05)	95/424	1.14 (0.84;1.54)	1.11 (0.82;1.51)
Quartile 3	39/424	1.30 (0.79;2.13)	1.37 (0.84;2.25)	78/424	0.94 (0.69;1.30)	0.97 (0.70;1.33)
Quartile 4	27/424	1 (reference)	1 (reference)	77/424	1 (reference)	1 (reference)
Per SD decrease	*181/1,695*	*1.15 (1.00;1.32)*	*1.09 (0.94;1.26)*	*376/1,695*	*1.10 (1.00;1.21)*	*1.10 (0.99;1.21)*

Values are hazard ratios with 95% confidence intervals.

The range of vasomotor reactivity for each quartile was as follows: quartile 1: 0–28.1 %/kPa, quartile 2: 28.1-39.3 %/kPa, quartile 3: 39.3-54.0 %/kPa, quartile 4: 54.0-229.3 %/kPa.

* Vasomotor reactivity was natural log transformed.

Model I: adjusted for age, sex and difference in blood pressure before and during vasomotor reactivity measurement.

Model II: adjusted for age, sex, current smoking, former smoking, use of blood pressure lowering medication, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, HDL-cholesterol, use of statins, history of vascular disease, carotid intima-media thickness, and difference in blood pressure before and during vasomotor reactivity measurement.

Abbreviations: n=number of deaths; N=number of people at risk; HR=hazard ratio; CI=confidence interval; SD=standard deviation; HDL=high-density lipoprotein.

Table 4. Vasomotor reactivity and the risk of stroke

Vasomotor reactivity*	**n/N**	**Model I HR (95% CI)**	**Model II HR (95% CI)**
Quartile 1	47/423	1.11 (0.72;1.71)	1.10 (0.71;1.70)
Quartile 2	46/424	1.15 (0.75;1.77)	1.15 (0.74;1.77)
Quartile 3	37/424	0.90 (0.57;1.43)	0.92 (0.58;1.45)
Quartile 4	38/424	1 (reference)	1 (reference)
Per SD decrease	*168/1,695*	*1.06 (0.91;1.23)*	*1.06 (0.91;1.23)*

Values are hazard ratios with 95% confidence intervals.

The range of vasomotor reactivity for each quartile was as follows: quartile 1: 0–28.1 %/kPa, quartile 2: 28.1-39.3 %/kPa, quartile 3: 39.3-54.0 %/kPa, quartile 4: 54.0-229.3 %/kPa.

* Vasomotor reactivity was natural log transformed.

Model I: adjusted for age, sex and difference in blood pressure before and during vasomotor reactivity measurement.

Model II: adjusted for age, sex, current smoking, former smoking, use of blood pressure lowering medication, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, HDL-cholesterol, use of statins, history of vascular disease, carotid intima-media thickness, and difference in blood pressure before and during vasomotor reactivity measurement.

Abbreviations: n=number of strokes; N=number of people at risk; HR=hazard ratio; CI=confidence interval; SD=standard deviation; HDL=high-density lipoprotein.

Table 5. Vasomotor reactivity and the risk of mortality, after censoring for incident stroke

Vasomotor reactivity*	n/N	All-cause mortality HR (95% CI)	n/N	Cardiovascular mortality HR (95% CI)
Quartile 1	150/423	1.49 (1.13;1.96)	41/423	1.76 (1.01;3.07)
Quartile 2	128/424	1.39 (1.05;1.83)	43/424	2.07 (1.20;3.56)
Quartile 3	103/424	1.13 (0.85;1.51)	28/424	1.32 (0.74;2.38)
Quartile 4	84/424	1 (reference)	19/424	1 (reference)
Per SD decrease	*465/1,695*	*1.12 (1.03;1.22)*	*131/1,695*	*1.20 (1.03;1.40)*

Values are hazard ratios with 95% confidence intervals.

The range of vasomotor reactivity for each quartile was as follows: quartile 1: 0–28.1 %/kPa, quartile 2: 28.1-39.3 %/kPa, quartile 3: 39.3-54.0 %/kPa, quartile 4: 54.0-229.3 %/kPa.

* Vasomotor reactivity was natural log transformed.

Models are adjusted for age, sex and difference in blood pressure before and during vasomotor reactivity measurement.

Abbreviations: n=number of deaths; N=number of people at risk; HR=hazard ratio; CI=confidence interval; SD=standard deviation.

higher risk of cardiovascular mortality since quartile 2 showed a stronger association than quartile 1.

After excluding the three participants with an exhausted vasomotor reactivity (<5.3%/kPa), effect sizes were similar (data not shown).

Interestingly, vasomotor reactivity was not associated with the risk of stroke (Table 4). Instead, the associations with both all-cause mortality and cardiovascular mortality remained significant after censoring for incident stroke (Table 5).

Our additional analyses showed no evidence for vasomotor reactivity being an intermediate between various cardiovascular risk factors and risk of mortality (Supplementary table 1).

Discussion

We found that people with a lower cerebral vasomotor reactivity have an increased risk of mortality. These associations were independent from cardiovascular risk factors and from incident stroke.

Strengths of this study are the population-based design; the thorough collection of events; and the long follow-up for both mortality and stroke. A potential limitation is selection bias because transcranial Doppler measurements failed in a large group of participants. The percentage of participants who died was significantly higher among the excluded participants, which indeed might point towards selection bias. Transcranial Doppler measurements failed mainly because of window failure, which occurred more often in women and in older participants.[18] Given that vasomotor reactivity decreases with age[26], coupled with higher risk

of cardiovascular disease with age, this could have led to a dilution of the effect. Another consideration is potential survivor effect as transcranial Doppler measurements were only performed at the second follow-up examination of the Rotterdam Study. It is possible that unhealthy people may have died during the intermediate time period. This would have resulted in an underestimation of the effect. A final remark is that we only assessed cIMT but did not measure the lumen of the carotid artery and were therefore not able to assess extracranial carotid artery stenosis. In the general population, the prevalence of moderate extracranial carotid artery stenosis ranges from 2.0-7.5% in people 60 years of age and older.[27] Given that it both affects cerebral vasomotor reactivity measurements and stroke, extracranial carotid artery stenosis could have influenced our results.[8]

We found that a lower vasomotor reactivity was associated with higher risk of mortality, especially cardiovascular mortality. These results suggest that a low vasomotor reactivity is a marker of accumulating vascular damage. We did find a dose-response relation between vasomotor reactivity and all-cause mortality, however this relation was not found for cardiovascular mortality. Moreover, no clear cut-off between normal and abnormal vasomotor reactivity values can be obtained from these analyses, as the cut-off points for the quartiles are based on this study population and cannot be generalized to other study populations. Vasomotor reactivity is measured in the cerebral vessels and previous studies have shown that in patients with carotid artery stenosis, those with a lower vasomotor reactivity have an increased risk of stroke.[8,11-16] Consequently, our findings with all-cause and cardiovascular mortality could be explained by stroke and stroke-related deaths. However, we did not find vasomotor reactivity to be associated with stroke. This is consistent with what was found before within the same study population, with shorter follow-up.[18] Furthermore, the association of lower vasomotor reactivity with both all-cause and cardiovascular mortality was independent from incident stroke. The main causes of cardiovascular death after censoring for strokes were heart failure, cardiac arrest, sudden death with unknown cause, and myocardial infarction. This supports the hypothesis that loss of cerebral vasomotor reactivity is a reflection of a more systemic dysfunction of the vascular system rather than only cerebrovascular damage. Further evidence comes from previous studies that have reported a link between peripheral artery endothelial dysfunction and cerebrovascular reactivity.[28,29] Still, we note that some studies did not find an association between flow-mediated vasodilatation (FMD) in the brachial artery, which is an indirect measure of peripheral endothelial dysfunction, and cerebrovascular reactivity.[30,31] Inconsistencies across studies might be explained by methodological differences and differences in study population.

Although associations with cardiovascular mortality attenuated after adjustment for cardiovascular risk factors and became statistically non-significant, an effect size in excess of 1.3 remained for each of the lowest three quartiles compared to the upper quartile. This suggests that part of the effect of vasomotor reactivity is independent from cardiovascular risk factors.

Also, it is questionable whether such an adjustment is a correction for potential confounders or actually an over-adjustment for possible intermediates of the causal chain. Nevertheless, some remarks can be made on the results after these adjustments. First, prior studies have shown that hypertension, smoking, and dyslipidemia disrupt the vascular homeostasis. This might cause endothelial dysfunction, which eventually contributes to cardiovascular disease.[3,32] Endothelial dysfunction leads to a lower excretion of dilatory factors, such as nitric oxide, and could therefore also lead to a lower vasomotor reactivity.[2] Second, it is possible that participants with unrecognized risk factors did not receive preventative treatment and therefore were more at risk for a cardiovascular event than those in whom cardiovascular risk factors were present and thus treated. This would lead to a minimal effect of adjusting for such risk factors. Third, we adjusted for baseline measurements of cardiovascular risk factors, which might be less representative for life-long exposure. A final consideration is that vasomotor reactivity reflects a different mechanism of vascular damage, not explained by cardiovascular risk factors, but by risk factors that we did not measure, such as genetic factors.[33,34] Conversely, we found that the associations of cardiovascular risk factors with mortality remained unchanged after adjusting for vasomotor reactivity. It is likely that these factors exert their effect through many different mediators, among which vasomotor reactivity.

Since vascular disease is the leading cause of mortality worldwide, there is an urgent need for preventive options. As such, markers of early vascular damage are of much interest. Our study on vasomotor reactivity and mortality is set in a community-dwelling population. Unlike in a clinical setting, our participants are relatively healthy and the amount of available in-depth data is limited. We therefore do not have additional data to clarify the potential cause of diminished vasomotor reactivity. The association between a lower vasomotor reactivity and higher risk of cardiovascular mortality thus merits further investigation. Specifically, unravelling the underlying mechanism and the possible contribution to identification of high-risk individuals is worthy of future research. Also, it would be of interest to investigate the association of vasomotor reactivity and white matter lesions measured on Magnetic Resonance Imaging (MRI).

In conclusion, our results indicate that loss of cerebral vasomotor reactivity is associated with an increased risk of mortality, especially cardiovascular mortality, independent of stroke. This suggests that impaired cerebral vasomotor reactivity reflects a systemically impaired vascular system.

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Supplementary table

Supplementary table 1. Associations between cardiovascular risk factors and all-cause mortality, before and after adjustment for vasomotor reactivity

	All-cause mortality n/N 557/1,695	
	Model I HR (95% CI)	Model II HR (95% CI)
Age	1.12 (1.10;1.13)	1.12 (1.10;1.13)
Sex	0.76 (0.61;0.94)	0.75 (0.60;0.93)
Systolic blood pressure	1.00 (0.99;1.00)	1.00 (0.99;1.00)
Diastolic blood pressure	1.00 (0.99;1.01)	1.00 (0.99;1.01)
Blood pressure lowering medication	1.17 (0.96;1.43)	1.16 (0.95;1.42)
Diabetes mellitus	1.20 (0.93;1.54)	1.18 (0.92;1.52)
Former smoking	1.16 (0.94;1.43)	1.17 (0.95;1.45)
Current smoking	2.08 (1.65;2.63)	2.09 (1.66;2.64)
Total cholesterol	0.93 (0.85;1.02)	0.93 (0.84;1.01)
HDL-cholesterol	0.86 (0.67;1.11)	0.87 (0.68;1.12)
Statins	0.72 (0.55;0.95)	0.72 (0.55;0.95)
History of vascular disease	1.55 (1.27;1.88)	1.52 (1.25;1.85)
Carotid intima-media thickness	1.84 (1.13;2.99)	1.81 (1.11;2.95)

Values are hazard ratios with 95% confidence intervals.

Model I: adjusted for age, sex, current smoking, former smoking, blood pressure lowering medication, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, HDL-cholesterol, use of statins, history of vascular disease, and carotid intima-media thickness.

Model II: adjusted for age, sex, current smoking, former smoking, use of blood pressure lowering medication, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, HDL-cholesterol, use of statins, history of vascular disease, carotid intima-media thickness, and vasomotor reactivity (natural log transformed).

Abbreviations: n=number of deaths; N=number of people at risk; HR=hazard ratio; CI=confidence interval; HDL=high-density lipoprotein.

Chapter 3.5

Insulin-like growth factor-I receptor stimulating activity is associated with dementia

Renée F.A.G. de Bruijn, Joseph A.M.J.L. Janssen, Michael P. Brugts, Cornelia M. van Duijn, Albert Hofman, Peter J. Koudstaal, M. Arfan Ikram

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Abstract

Background: Insulin-like growth factor-I (IGF-I) is a pleiotropic hormone. Several studies have related IGF-I levels to dementia, but evidence remains inconclusive. IGF-I receptor stimulating activity is a more direct measure of biologically available IGF-I than total IGF-I levels. We investigated whether IGF-I receptor stimulating activity is associated with prevalent and incident dementia.

Methods: IGF-I receptor stimulating activity was measured using an IGF-I kinase receptor activation assay in 1,014 people from the Rotterdam Study. Dementia was assessed at baseline (1997-1999) and continuously during follow-up until September 2011. Associations of IGF-I receptor stimulating activity with prevalent dementia were investigated using logistic regression and with incident dementia using Cox proportional hazards models. All models were adjusted for age and sex, and additionally for hypertension, glucose, waist circumference, *APOE*-ε4 carrier status, total cholesterol, and HDL-cholesterol.

Results: Thirty participants had prevalent dementia and during 8,589 person-years of follow-up 135 people developed incident dementia. A higher level of IGF-I receptor stimulating activity was associated with a higher prevalence of dementia (fully adjusted odds ratio 1.47, 95% CI 1.10;1.97) and with higher risk of incident dementia (fully adjusted hazard ratio 1.15, 95% CI 1.00;1.33). Similar associations were found for Alzheimer disease and in people without diabetes mellitus.

Conclusions: Higher levels of IGF-I receptor stimulating activity are associated with a higher prevalence and with a higher incidence of dementia. These results suggest that IGF-I increases in response to neuropathological changes in dementia and could reflect a state of IGF-I resistance in dementia.

Introduction

Dementia is a multifactorial disease in which amyloid β, tau, and vascular pathology accumulate and interact leading to loss of neuronal tissue. Given that neuronal tissue loss is an important aspect of dementia, much emphasis has been put on the role of insulin-like growth factor-I (IGF-I) in dementia.[1] IGF-I is a hormone that regulates cell metabolism and closely interacts with insulin and growth hormone to stimulate tissue growth. Animal studies have shown that in the brain IGF-I stimulates neurogenesis, reduces amyloid β burden, and influences tau phosphorylation.[2-4] These findings suggest that IGF-I levels might be altered in dementia, but the effect of IGF-I in humans remains inconclusive.[5-10] Some studies found lower levels of IGF-I in dementia or cognitive decline.[8-10] Hence, it is suggested that low IGF-I levels lead to less protection against neuropathological changes.[8,9] Yet, other studies found higher levels of IGF-I in dementia and propose that IGF-I levels might alter in response to neuropathological changes in dementia.[5-7]

A major difficulty in research on IGF-I is that most of the circulating IGF-I is bound to insulin-like growth factor binding proteins (IGFBPs) and therefore biologically inactive. Consequently, levels of total IGF-I, as measured in previous studies, poorly reflect the actual IGF-I bioactivity.[5-10] A recently developed IGF-I specific kinase receptor activation assay (KIRA) has enabled assessment of the IGF-I receptor stimulating activity and thus the actual IGF-I bioactivity.[11,12] IGF-I receptor stimulating activity measured using KIRA was only modestly correlated to other parameters of the IGF-I system, such as total serum IGF-I level and IGF-I/IGFBP-3 ratio.[13] Hence, KIRA reveals additional information about circulating IGF-I and may facilitate better understanding of the role of IGF-I in dementia.

In an elderly, population-based cohort, we studied the association of circulating IGF-I receptor stimulating activity with both prevalent and incident dementia.

Materials and Methods

Setting

This study was embedded within the prospective, population-based Rotterdam Study, which aims to study risk factors and determinants of chronic diseases in the elderly. The Rotterdam Study started in 1990 among inhabitants of 55 years and older residing in Ommoord, a district of Rotterdam, the Netherlands. Of the 10,215 invited inhabitants, 7,983 agreed to participate in the baseline examinations. Up until 2014, there have been four follow-up examinations. Details of the study have been described elsewhere.[14] For the current study, the second follow-up examination between 1997-1999 was used as baseline, because IGF-I receptor stimulating activity was measured in blood samples collected at that visit.

The medical ethics committee at Erasmus University of Rotterdam approved the study and written informed consent was obtained from all participants.

Assessment of IGF-I receptor stimulating activity

IGF-I receptor stimulating activity levels were measured using an IGF-I kinase receptor activation assay (KIRA) (intra- and inter-assay coefficients of variation of 5.2 and 12.2%, respectively; cross-reactivity of 15% for IGF-II).[12] We measured IGF-I bioactivity in individual serum samples in vitro under physiological conditions. The principle of IGF-I KIRA assay is based on measurement and quantification of the IGF mediated phosphorylation of the IGF-I receptor, which normally occurs after binding of IGF-I to the IGF-I receptor. To conduct the IGF-I KIRA assay, a human embryonic cell line is transfected with a copy DNA of the full length human IGF-I receptor. When serum of an individual is added to intact cells seeded in 48-well microtiter plates, the tyrosine residues of the beta-subunits of the IGF-I receptor will become phosphorylated by the IGF-I present in this serum sample.[15] The IGF-I KIRA assay takes into account the modifying effect of IGFBPs on the interaction between IGF-I and the IGF-I receptor. A more extensive explanation of the method has been described previously.[11,16] Of the 5,990 participants that were alive in 1997-1999, 4,797 people participated in the examination used as baseline for this study. IGF-I receptor stimulating activity levels were measured in blood samples of 1,050 randomly selected participants due to financial constraints. Five participants were excluded because their blood samples were not correctly matched and 14 because measurements repeatedly did not pass prior defined assay acceptance criteria (inter-assay coefficient of variation <10%). Another 17 participants were excluded because they did not undergo sufficient screening for dementia. Eventually, 1,014 participants were included in the analyses.

Assessment of dementia

Participants were screened for dementia at baseline and follow-up examinations using a three-step protocol. Screening was done using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level.[17,18] Screen-positives (MMSE <26 or GMS organic level >0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[19] Participants who were suspected of having dementia, underwent, if necessary, further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. When information on neuro-imaging was required and available, it was used for decision making on the diagnosis. For all suspected cases of dementia, a consensus panel, led by a neurologist,

decided on the final diagnosis in accordance with standard criteria for dementia (DSM-III-R) and Alzheimer disease (NINCDS-ADRDA).[20,21] Follow-up for incident dementia was virtually complete (97.8%) until September 2, 2011.

Other measurements

Information on *apolipoprotein E* (*APOE*) genotype was obtained using polymerase chain reaction on coded DNA samples without knowledge of the dementia diagnosis. *APOE*-ε4 carrier status was defined as carrier of one or two ε4 alleles Blood pressure was calculated as the average of two measurements at the right brachial artery using a random-zero sphygmomanometer with the participant in a sitting position. Hypertension was defined as a blood pressure ≥140/90 mmHg or use of blood pressure lowering medication, prescribed for the indication of hypertension. Waist circumference was measured in centimeters at the research center visit. Diabetes mellitus was defined as a fasting serum glucose level ≥7.0 mmol/L, non-fasting serum glucose level ≥11.1 mmol/L, or use of anti-diabetic medication. Serum glucose, total cholesterol, and high-density lipoprotein (HDL)-cholesterol levels were acquired by an automated enzymatic procedure (Boehringer Mannheim System). Missing values in covariates (for *APOE*-ε4 carrier status 4.8%, for all other covariates less than 3.5%) were imputed based on age and sex.

Statistical analyses

We examined whether the risk of dementia was different in people with higher levels of IGF-I receptor stimulating activity than in people with lower levels using two different epidemiological designs to obtain the relative risk. In the cross-sectional analyses, we used logistic regression models to assess the association between IGF-I receptor stimulating activity and prevalent dementia. In the longitudinal analyses, we used Cox proportional hazards models to assess the association between IGF-I receptor stimulating activity and incident dementia. IGF-I receptor stimulating activity was entered per standard deviation (SD) into the models. We also studied IGF-I receptor stimulating activity in tertiles, taking the lowest tertile as reference. All models were adjusted for age and sex (Model I) and additionally for hypertension, glucose, waist circumference, *APOE*-ε4 carrier status, total cholesterol, and HDL-cholesterol (Model II) for being potential confounders. The underlying time-scale in the Cox proportional hazards models was the follow-up time, which was defined from time at blood sample collection (1997-1999) until September, 2011. Subjects were censored within this time period when they were diagnosed with dementia, died, or decided to terminate their participation in the study.

IGF-I receptor stimulating activity is significantly lower in subjects with diabetes mellitus and might be influenced by anti-diabetic medication.[12] Therefore, we repeated our analyses after

excluding participants with prevalent diabetes mellitus (n=109) and participants who did not have information on fasting serum glucose level or use of anti-diabetic medication (n=22).
We separately investigated the association between IGF-I receptor stimulating activity and Alzheimer disease.
Analyses were performed using IBM SPSS statistics version 20.0 (IBM Corp, Armonk, NY, USA).

Results

Baseline characteristics of the study population are provided in table 1. At baseline, 30 participants suffered from prevalent dementia, of whom 23 had Alzheimer disease. During a follow-up of 8,589 person-years (mean follow-up of 8.7 years, SD 3.4 years), 135 participants developed dementia, of whom 109 Alzheimer disease.
People with a higher level of IGF-I receptor stimulating activity had a higher prevalence of dementia than people with a lower level of IGF-I receptor stimulating activity, especially in fully adjusted models: odds ratio (OR) per SD increase in IGF-I receptor stimulating activity 1.47 (95% confidence interval (CI) 1.10;1.97) (Table 2). Similar associations were observed for Alzheimer disease (OR 1.41, 95% CI 1.02;1.96) and after restriction to people without diabetes mellitus (OR 1.42, 95% CI 1.00;2.02). We also found that people with a higher IGF-I receptor stimulating activity had an increased risk of dementia, especially in fully adjusted models: hazard ratio (HR) per SD increase in IGF-I receptor stimulating activity 1.15 (95% CI 1.00;1.33) (Table 3). Again, results remained largely unchanged for Alzheimer disease (HR

Table 1. Baseline characteristics

	Prevalent dementia N=30	**At risk for incident dementia N=984**
Age, years	81.5 (8.3)	72.0 (7.1)
Females	70.0%	55.8%
IGF-I receptor stimulating activity, pmol/L	208.1 (77.6)	179.1 (55.5)
Apolipoprotein E-ε4 carrier status	71.4%	27.4%
Hypertension	82.1%	75.5%
Waist circumference, cm	94.2 (8.3)	93.8 (11.1)
Glucose, mmol/L	6.03 (1.07)	6.01 (1.51)
Total cholesterol, mmol/L	5.52 (1.17)	5.83 (1.00)
HDL-cholesterol, mmol/L	1.34 (0.46)	1.38 (0.37)

Data are presented as means (standard deviations) or percentages
Abbreviations: N=number of people; IGF-I=insulin-like growth factor-I; HDL=high-density lipoprotein

Table 2. IGF-I receptor stimulating activity and prevalence of dementia

	Dementia		Alzheimer disease	
	OR[a] (95% CI) n/N 30/984	OR[b] (95% CI) n/N 23/860	OR[a] (95% CI) n/N 23/984	OR[b] (95% CI) n/N 18/860
Model I	1.28 (0.97;1.68)	1.20 (0.87;1.67)	1.22 (0.89;1.66)	1.20 (0.84;1.72)
Model II	1.47 (1.10;1.97)	1.42 (1.00;2.02)	1.41 (1.02;1.96)	1.44 (0.99;2.09)

Values represent odds ratios (95% confidence interval) per standard deviation increase in IGF-I receptor stimulating activity.

[a] Including participants with prevalent diabetes mellitus.

[b] Excluding participants with prevalent diabetes mellitus.

Model I: adjusted for age and sex.

Model II: adjusted for age, sex, hypertension, glucose, waist circumference, *apolipoprotein E*-ε4 carrier status, total cholesterol, and high-density lipoprotein-cholesterol.

Abbreviations: IGF-I=insulin-like growth factor-I; OR=odds ratio; CI=confidence interval; n=number of cases; N=number of controls.

Table 3. IGF-I receptor stimulating activity and risk of incident dementia

	Dementia		Alzheimer disease	
	HR[a] (95% CI) n/N 135/984	HR[b] (95% CI) n/N 117/860	HR[a] (95% CI) n/N 109/984	HR[b] (95% CI) n/N 97/860
Model I	1.13 (0.98;1.31)	1.13 (0.95;1.34)	1.14 (0.98;1.33)	1.13 (0.94;1.35)
Model II	1.15 (1.00;1.33)	1.16 (0.98;1.37)	1.17 (1.00;1.37)	1.17 (0.97;1.40)

Values represent hazard ratios (95% confidence interval) per standard deviation increase in IGF-I receptor stimulating activity.

[a] Including participants with prevalent diabetes mellitus.

[b] Excluding participants with prevalent diabetes mellitus.

Model I: adjusted for age and sex.

Model II: adjusted for age, sex, hypertension, glucose, waist circumference, *apolipoprotein E*-ε4 carrier status, total cholesterol, and high-density lipoprotein-cholesterol.

Abbreviations: IGF-I=insulin-like growth factor-I; HR=hazard ratio; CI=confidence interval; n=number of cases; N=number of people at risk.

1.17, 95% CI 1.00;1.37) and after restriction to people without diabetes mellitus (HR 1.16, 95% CI 0.98;1.37).

Figure 1 shows the unadjusted cumulative incidence curves of dementia per tertile of IGF-I receptor stimulating activity. People in the two highest tertiles of IGF-I receptor stimulating activity had a higher risk of dementia than people in the lowest tertile. The corresponding fully adjusted hazard ratios were: tertile 2 compared to tertile 1, 1.44 (95% CI 0.94;2.22) and tertile 3 compared to tertile 1, 1.50 (95% CI 0.98;2.29).

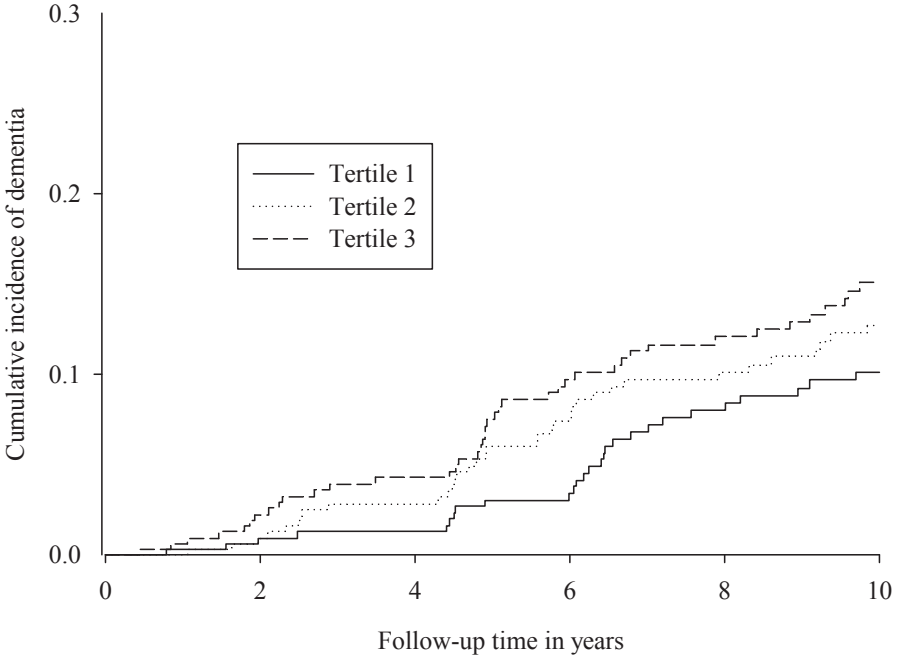

Figure 1. Cumulative incidence of dementia per tertile of IGF-I receptor stimulating activity
Cumulative incidence curves of dementia per tertile of IGF-I receptor stimulating activity. Tertile 1 represents the lowest tertile of IGF-I receptor stimulating activity, tertile 3 the highest.

Discussion

We found that a higher level of IGF-I receptor stimulating activity was associated with both a higher prevalence and incidence of dementia, including Alzheimer disease.

There are several potential explanations for higher levels of IGF-I receptor stimulating activity in dementia. Firstly, due to neuropathological changes in dementia, there is loss of neuronal tissue. Consequently, such tissue loss could lead to increased IGF-I production in order to stimulate neurogenesis.[2] Secondly, animal studies have shown that IGF-I reduces amyloid β burden and influences tau phosphorylation, two key processes associated with the etiology of dementia.[3,4] Hence, IGF-I levels might be higher in dementia to overcome these neuropathological processes. Finally, other processes involved in dementia, such as inflammation, might cause loss of sensitivity of the IGF-I receptor.[1] Therefore, higher IGF-I levels in dementia could reflect a state of IGF-I resistance in dementia. In summary, our results suggest that IGF-I levels increase in response to neuropathological changes in dementia.

Our findings, that higher IGF-I receptor stimulating activity is associated with a higher prevalence and risk of dementia, are supported by several studies.[5-7] Yet, other studies found

that lower levels of serum total IGF-I were related to a higher prevalence of dementia or to more cognitive decline.[8-10] Most studies only assessed total serum IGF-I levels or used indirect methods to measure the total biological available IGF-I concentration. We used KIRA, which has been validated to reliably measure the concentration of biological active and receptor stimulating IGF-I.[11,12]

An additional inconsistency across studies is the severity of dementia. For example, one study found higher levels of circulating total IGF-I in demented participants compared to healthy controls, while another study found an opposite association.[5,8] A large difference between these two studies was the MMSE-score of the demented participants, which was much higher in the first study. It is possible that in early, less severe stages of dementia IGF-I levels are increased as a response to overcome disease-associated IGF-I resistance, whereas they decrease in the more advanced and severe stages of the disease.[1,5] Liver cells producing IGF-I could become exhausted and ultimately fail to produce IGF-I leading to depletion.[1,5] In our prevalent cases, we also diagnosed dementia at a relatively early stage, with a mean MMSE-score of 18.

Strengths of this study are its prospective, population-based design and use of a direct measure of IGF-I receptor stimulating activity. The prospective design enabled us to not only investigate the association with prevalent dementia, but also with incident dementia. Another strength is the thorough dementia case finding. Since follow-up for dementia was virtually complete (97.8%), we are confident there was no selective loss of demented participants. Our study also has limitations. Firstly, IGF-I receptor stimulating activity was measured in peripheral blood samples. Although circulating IGF-I crosses the blood-brain barrier, we could not assess to what extent our measurements reflect IGF-I receptor stimulating activity in the brain.[22] Secondly, no total serum IGF-I levels were available and therefore we were not able to compare associations. Thirdly, IGF-I receptor stimulating activity was measured at the second follow-up of this study, which could have led to some survivor effect. A final consideration is that the results of our cross-sectional analysis were based on a limited number of 30 cases. This might be an explanation why we found a stronger effect of higher IGF-I receptor stimulating activity levels in the cross-sectional analysis than in the longitudinal analysis, especially in the fully adjusted models. It is possible that the fully adjusted model was over fitted in the cross-sectional analysis due to the small number of cases.

In conclusion, we found that a higher level of IGF-I receptor stimulating activity was associated with a higher prevalence of dementia and to a higher incidence of dementia. These results suggest that IGF-I increases in response to the neuropathological changes in dementia and could reflect a state of IGF-I resistance in dementia.

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Chapter 4

Behavioral and emotional factors

Chapter 4.1

The association between physical activity and dementia in an elderly population

Renée F.A.G. de Bruijn, Elisabeth M.C. Schrijvers,
Karen A. de Groot, Jacqueline C.M. Witteman, Albert Hofman, Oscar H. Franco,
Peter J. Koudstaal, M. Arfan Ikram

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Abstract

Background: Several studies have associated physical activity with the risk of dementia, but mostly did so during short follow-up. It remains unclear whether physical activity also affects dementia during longer follow-up. We examined the association between physical activity and risk of dementia during a follow-up period up to 14 years.

Methods: From 1997-1999, physical activity was assessed using a validated questionnaire in 4406 elderly people (age range 61-97) from the prospective population-based Rotterdam Study. Follow-up for dementia was complete until January 1, 2011. We used Cox proportional hazards models to assess the association between physical activity and incident dementia. Next, we stratified follow-up time using a cut-off of 4 years. We separately investigated dementia due to Alzheimer disease.

Results: During 38,631 person-years, 583 participants developed dementia. When adjusting for age and sex, we found a borderline significant association between higher physical activity and lower risk of dementia (HR 0.95, 95% CI 0.87;1.04). This association was confined to follow-up up to 4 years (HR 0.82, 95% CI 0.71;0.95), and not to follow-up of at least 4 years (HR 1.04, 95% CI 0.93;1.16). Additional adjustments only slightly attenuated the associations. A similar pattern was present for Alzheimer disease.

Conclusions: We found a higher level of physical activity to be associated with a lower risk of dementia. This association was confined to follow-up for up to 4 years and not to longer follow-up, suggesting either a role for reverse causality or only a short term effect of late-life physical activity in an elderly population.

Introduction

The number of patients with dementia, and especially Alzheimer disease, is rapidly increasing, indicating an urgent need to develop preventive interventions. Consequently, one of the main areas of research is to identify modifiable risk factors for dementia and Alzheimer disease.[1]
Emerging evidence suggests that physical activity may have a protective effect on the risk of dementia.[2,3] Physical activity is known to have an inverse association with several chronic diseases, such as cardiovascular disease and diabetes.[4,5] Since risk factors and pathways associated with cardiovascular disease also seem to play a role in the pathogenesis of dementia, physical activity may have a protective effect for dementia too.[6] Alternatively, being physically active could directly reduce the risk of dementia by improving cerebral perfusion and increasing neurogenesis.[7,8]
Several epidemiological studies have associated a higher level of physical activity with a reduced risk of dementia or cognitive decline.[9-24] Most of these studies had relatively short follow-up.[9-12,14,18-22] Studies with long follow-up periods have yielded inconsistent results.[24,25] Therefore, it remains unclear whether physical activity also affects dementia during longer follow-up. An important consideration when investigating lifestyle risk factors, such as physical activity, is that deterioration in these factors could also be part of the pre-diagnostic phase of dementia, where cognitive symptoms are present but a diagnosis of dementia has not yet been assigned. A longer follow-up can also provide a solution for such reverse causality, because people with shorter follow-up who may be suffering from pre-diagnostic disease can be excluded.
Therefore, the aim of our study was to examine the association of physical activity with dementia, and separately with Alzheimer disease, during longer follow-up. We specifically sought to assess the effect of pre-diagnostic dementia by excluding all people with shorter follow-up.

Methods

Setting

This study was conducted within the Rotterdam Study, a prospective population-based cohort study that started in 1990 and is conducted among inhabitants, aged 55 years and older, of Ommoord, a suburb of Rotterdam, the Netherlands. Of the 10,274 eligible inhabitants, 7,893 agreed to participate in the baseline examinations. Up until 2012, there have been 4 follow-up examinations. Details of the study have been described elsewhere.[26] For the current study, the second follow-up examination from 1997-1999 was used as baseline, because data on physical activity were collected only at that follow-up visit.

The medical ethics committee at Erasmus University of Rotterdam approved the study and written informed consent was obtained from all participants.

Physical activity

Physical activity data was assessed by means of an adapted version of the Zutphen Physical Activity Questionnaire.[27] The Zutphen Physical Activity Questionnaire has been validated previously: the test-retest reliability was 0.93 and the correlation with doubly labeled water, the golden standard measurement of physical activity, was 0.61.[28] The original questionnaire contains questions on walking, cycling, gardening, diverse sports, and hobbies. To obtain a more complete overview of physical activity, questions on housekeeping activities were added. Participants were asked how many hours they spent per week on these activities during the past two weeks. For some activities, like sports and gardening, participants were asked whether this activity was practised only during summer or winter. When answered positive, the given period of time for that activity was divided by two. To study body movement associated with the activity, we used the metabolic equivalent of task (MET). MET is a widely used method to assign intensity units to physical activity questionnaires.[29] The MET-value is based on the ratio of work metabolic rate to resting metabolic rate and 1 MET is defined as 1 kcal/kg/hour. The number of MET-hour for an individual spent on a specific activity was therefore calculated by multiplying the MET-value by time spent on that specific activity (in hours) per week.[29]

Of the 5,990 participants that were alive in 1997-1999, 4,797 people participated in the examination used as baseline for this study. A total of 4,559 participants completed physical activity data collection and underwent screening for dementia. Fifty-one participants were excluded due to unreliable completion of physical activity data collection. Furthermore, 102 participants were excluded because of prevalent dementia. Eventually, 4,406 subjects were included in the analyses.

Assessment of dementia

Participants were screened for dementia at baseline and follow-up examinations using a three-step protocol. Screening was done using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level.[30,31] Screen-positives (MMSE <26 or GMS organic level >0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[32] Participants who were suspected of having dementia, underwent, if necessary, further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. When informa-

tion on neuro-imaging was required and available, it was used for decision making on the diagnosis. In the end, a consensus panel, led by a neurologist, decided on the final diagnosis in accordance with standard criteria using the DSM-III-R criteria for dementia and the NINCDS-ADRDA for Alzheimer disease.[33,34] Follow-up for incident dementia was virtually complete (97.5%) until January 1, 2011.

Other measurements

Low educational level was defined as primary education only or primary education with an unfinished higher education, which means less than 12 years of education. Smoking habits were assessed by an interview asking participants whether they were currently smoking cigarettes, cigars or pipe. Information on *APOE* genotype was obtained using polymerase chain reaction on coded DNA samples without knowledge of the dementia diagnosis.[35] Blood pressure was calculated as the average of two measurements at the right brachial artery using a random-zero sphygmomanometer with the participant in a sitting position. Hypertension was defined as a blood pressure ≥ 140/90 mmHg or use of blood pressure lowering medication, prescribed for the indication of hypertension. Weight and height were measured at the research center visit. Body mass index (BMI) was calculated dividing weight in kilograms by height in meters squared. When measurements for weight or height were missing for the baseline examination of this study (11.3%), we used measurements from previous follow-up examinations to calculate BMI. Diabetes mellitus was defined as a fasting serum glucose level ≥ 7.0 mmol/L or use of glucose lowering drugs. Serum glucose, total cholesterol, and HDL-cholesterol levels were acquired by an automated enzymatic procedure (Boehringer Mannheim System). Remaining missing values in covariates (overall 5.7 %) were imputed based on age and sex.

Statistical analyses

Because of a right skewed distribution of physical activity, we first performed a natural logarithmic transformation. After obtaining a roughly normal distribution of the data, physical activity was entered continuously per standard deviation (SD) increase into the models.
We investigated the associations between physical activity and incident dementia using Cox proportional hazards. The underlying time-scale in these models was the follow-up time. Follow-up time was defined from time at physical activity assessment until January 1, 2011. Subjects were censored within this time period when they were diagnosed with dementia, died, or decided to terminate their participation in the study. All models were adjusted for age in single years and sex. We adjusted subsequently for score on MMSE at baseline, low educational level, smoking, and *APOE*-ε4 carrier status (Model II) for being potential confounders and for hypertension, BMI, diabetes, total cholesterol, and HDL-cholesterol (Model III) for

being potential intermediate factors. The same analyses were repeated examining the effect of physical activity on the risk of Alzheimer disease.
In order to investigate the possible effect of reverse causality, we expanded our analyses by stratifying the follow-up time into a follow-up period up to 4 years, and a follow-up period of at least 4 years until the end of the study period. We decided to use 4 years as cut-off, as there is no real consensus on the average length of the pre-diagnostic phase, and 4 years was the overall average of follow-up time in previous studies. However, this cut-off is arbitrary and we therefore conducted a sensitivity analysis where we subsequently used cut-offs of 2, 3, 5, and 6 years, as this was the range of follow-up time in most studies.[9-12,14,18-22]
All analyses were done using the SPSS statistical package 17.0 (SPSS inc., Chicago, Illinois).

Results

The baseline characteristics of the study population are presented in table 1. After a mean follow-up of 8.8 years, 583 people were diagnosed with incident dementia and 490 with incident Alzheimer disease. Of the incident dementia cases, 149 were diagnosed within 4 years, and 434 after 4 years of follow-up. With regard to Alzheimer disease, these numbers were 135 and 355, respectively. The mean ages at onset of dementia in these two strata slightly differed:

Table 1. Baseline characteristics of the study population

	Study population N = 4,406
Age, years	72.7 (7.2)
Female, %	59.0
Leisure time physical activity, MET-hours	75.5 (49.0 – 109.7)
APOE-ε4 allele present, %	27.3
Low educational level, %	31.2
Current smoking, %	18.5
Score on MMSE, points	27.7 (1.9)
Hypertension, %	78.6
Serum total cholesterol, mmol/L	5.83 (0.98)
Serum HDL-cholesterol, mmol/L	1.39 (0.40)
BMI, kg/m^2	26.9 (4.0)
Diabetes, %	10.0

Data are presented as means (standard deviations) or percentages.
[a]Median (interquartile range) presented because of skewed distribution.
Abbreviations: *APOE=apolipoprotein E*; BMI=body mass index; HDL=high-density lipoprotein; MET=metabolic equivalent of task; MMSE=Mini-Mental State Examination.

Table 2. Associations between physical activity and incident dementia

	Dementia n/N = 583/4,406 HR (95% CI)	Alzheimer disease n/N = 490/4,406 HR (95% CI)
Model I	0.95 (0.87;1.04)	1.01 (0.91;1.11)
Model II	0.94 (0.86;1.03)	0.99 (0.90;1.09)
Model III	0.93 (0.85;1.02)	0.98 (0.89;1.08)

Values are expressed per standard deviation increase in natural log transformed physical activity.
Model I: adjusted for age and sex.
Model II: adjusted for age, sex, score on MMSE, low educational level, smoking, and *APOE*-ε4 carrier status.
Model III: adjusted for age, sex, score on MMSE, low educational level, smoking, *APOE*-ε4 carrier status, hypertension, BMI, diabetes, total cholesterol, and HDL-cholesterol.
Abbreviations: *APOE=apolipoprotein E*; BMI=body mass index; CI=confidence interval; HDL=high-density lipoprotein; HR=hazard ratio; MMSE=Mini-Mental State Examination; n=number of cases; N=number of people at risk.

81.8 years (SD 6.4, age range 65.6-95.7) up to 4 years of follow-up, and 83.4 years (SD 6.1, age range 69.0-102.3) after 4 years of follow-up.

Table 2 shows the associations between physical activity and incident dementia. We only found a borderline significant effect of physical activity on incident dementia in the total follow-up period (hazard ratio (HR) of dementia per SD increase in physical activity 0.95, 95% CI 0.87;1.04). Additional adjustments for potential confounders (Model II) and potential intermediate factors (Model III) only slightly altered the associations. Table 2 also shows that we found a similar association between physical activity and Alzheimer disease.

Table 3 presents the results of the models using the two strata of follow-up. There was a significant association between higher physical activity and lower risk of incident dementia

Table 3. Associations between physical activity and dementia, with stratified follow-up time

	Dementia		Alzheimer disease	
	Follow-up ≤ 4 yrs n/N = 149/4,406 HR (95% CI)	Follow-up > 4 yrs n/N = 434/3,771 HR (95% CI)	Follow-up ≤ 4 yrs n/N = 135/4,406 HR (95% CI)	Follow-up > 4 yrs n/N = 355/3,771 HR (95% CI)
Model I	0.82 (0.71;0.95)	1.04 (0.93;1.16)	0.87 (0.74;1.02)	1.10 (0.97;1.25)
Model II	0.83 (0.72;0.96)	1.01 (0.90;1.13)	0.88 (0.76;1.03)	1.07 (0.94;1.21)
Model III	0.84 (0.73;0.97)	1.00 (0.89;1.12)	0.89 (0.76;1.03)	1.05 (0.93;1.20)

Values are expressed per standard deviation increase in natural log transformed physical activity.
Model I: adjusted for age and sex.
Model II: adjusted for age, sex, score on MMSE, low educational level, smoking, and *APOE*-ε4 carrier status.
Model III: adjusted for age, sex, score on MMSE, low educational level, smoking, *APOE*-ε4 carrier status, hypertension, BMI, diabetes, total cholesterol, and HDL-cholesterol.
Abbreviations: *APOE=apolipoprotein E*; BMI=body mass index; CI=confidence interval; HDL=high-density lipoprotein; HR=hazard ratio; MMSE=Mini-Mental State Examination; n=number of cases; N=number of people at risk.

Table 4. Associations between physical activity and dementia, with follow-up time stratified using cut-offs ranging from 2 to 6 years

	Dementia			
Cut-off point of follow-up time	n/N	Short follow-up HR (95% CI)	n/N	Long follow-up HR (95% CI)
2 Years	76/4,406	0.78 (0.65;0.95)	507/4,096	1.00 (0.91;1.11)
3 Years	136/4,406	0.84 (0.72;0.98)	447/3,928	1.02 (0.91;1.14)
5 Years	217/4,406	0.89 (0.78;1.02)	366/3,547	1.01 (0.89;1.14)
6 Years	285/4,406	0.93 (0.83;1.05)	298/3,325	0.99 (0.86;1.13)

Values are expressed per standard deviation increase in natural log transformed physical activity.
All models are adjusted for age and sex.
Abbreviations: CI=confidence interval; HR=hazard ratio; n=number of cases; N=number of people at risk.

with follow-up up to 4 years (HR adjusted for age and sex 0.82, 95% CI 0.71;0.95), but not with follow-up from 4 years until the end of follow-up. After additional adjustments for potential confounders (Model II) and intermediate factors (Model III), the association during the follow-up period up to 4 years remained significant. We also found a similar pattern of associations when we studied the risk of Alzheimer disease separately, though non-significant (Table 3).
The sensitivity analysis, using subsequent cut-offs of 2, 3, 5, and 6 years, consistently showed a stronger association in the shorter follow-up periods compared to the longer follow-up periods (Table 4). Interestingly though, the later the cut-off the less significant the association in the shorter follow-up period became.

Discussion

We found, in an elderly population, that a higher level of physical activity was associated with a lower risk of dementia, but only with a follow-up period up to 4 years and not with a follow-up period of at least 4 years. These associations remained significant after adjustments for potential confounders. A similar pattern was found for Alzheimer disease, albeit non-significant.

An emerging body of literature has reported on an inverse relation between physical activity and the risk of dementia or cognitive decline.[9-24] Similar to our study, most of these previous studies used questionnaires to assess physical activity. Two prospective studies used alternative approaches and therefore warrant further mentioning. Using doubly labeled water, which is the golden standard for total physical activity, one study found a protective effect of physical activity on cognitive decline.[19] The second study, which used actigraphy to obtain physical

activity data, found physical activity to be inversely associated with the risk of Alzheimer disease.[9]
An important point to note in previous work is that most studies had follow-up of 2 to 6 years.[9-12,14,18-22] Given the long pre-clinical phase of dementia, there is a possibility that pre-diagnostic disease may have affected these results. The main novelty of our study is that we tried to disentangle the effect of pre-diagnostic disease by stratifying the follow-up time. We found a strong, significant association only with a follow-up up to 4 years and not with a follow-up after 4 years. This suggests that pre-diagnostic disease during shorter follow-up leading to reverse causality may indeed play a role when assessing physical activity. Further evidence for this issue comes from a recently published meta-analysis, which concluded that a significant part of the inconsistent results between the 21 studies analyzed, was due to difference in length of follow-up.[25]

A second explanation for our data might be that physical activity has a true protective effect on the risk of dementia, but only on the short term. Intervention studies have indeed found a positive effect of physical activity on cognition in subjects at high risk for dementia.[36,37] However, like most observational studies, these studies also had a relatively short follow-up. It could be that a physically active lifestyle delays clinical symptoms of dementia. Several studies have suggested that people with a more engaged and active lifestyle, are able to endure more neuropathological changes, like neurofibrillary tangles and senile plaques, before they suffer from the clinical symptoms of dementia.[38] Because we only measured physical activity levels once, it could be that higher levels of physical activity delayed clinical onset for people that were already on the verge of developing dementia.
Previous studies have also suggested that cardiovascular risk factors might act as an intermediate factor when examining the association between physical activity and dementia.[2,3] In our study, we were not able to support this hypothesis, since the associations were similar after adjusting for several cardiovascular risk factors.

A final point to consider is that although we did not find an association during longer follow-up this does not mean that physical activity does not have a beneficial effect in the long term. A large, population based study with a mean follow-up of 21 years, found physical activity to be associated with a lower incidence of dementia and Alzheimer disease.[24] A major difference with our study, though, is the age of the participants at time of assessment of physical activity. While our participants were elderly people with a mean age at baseline of 73 years, their study participants were middle aged people. It could be that assessing physical activity at midlife is less prone to misclassification over time, since elderly are more susceptible to illness and disability and therefore might change their lifestyle more rapidly.
The absence of association over longer follow-up in our study may therefore be explained by

the possibility that participants altered their physical activity levels, resulting in a less representative physical activity baseline measurement over longer follow-up. Besides the effect of aging, there is also a possibility that a longer exposure to a physically active lifestyle is needed to assess a beneficial effect on the risk of late-life dementia. Since the neuropathological changes leading to dementia and Alzheimer disease may occur long before the clinical diagnose of dementia, it could be that physical activity only protects against development of late-life dementia on middle-age, but that at a later age there is no causal relation anymore.[39] A similar pattern is seen for several cardiovascular risk factors.[40,41] However, more research is required before firm conclusions can be drawn.

Strengths of the current study are its prospective population based-design, large sample size and relatively long follow-up period. Since follow-up was nearly complete for dementia case finding, we are confident that there was no selective loss to follow-up of demented participants.[42] Because of the length of our follow-up period, we were able to stratify follow-up time and assess differences between shorter and longer follow-up when examining the association between physical activity and dementia.

Our study also has some limitations that need to be mentioned. Because we used a questionnaire to assess physical activity measurement, we had to rely on subjective participant information, which is prone to misclassification. Moreover, the questionnaire did not contain questions on work related physical activity. Although this can also be an important source of physical activity, our study population had an age range of 61-97; hence most participants were retired or did not have a paid job (95.6%). Finally, there might be some survivor effect as physical activity was assessed at the second follow-up exam of the Rotterdam Study and unhealthy people may have died during the intermediate time period.

In conclusion, we found a higher level of physical activity to be associated with a lower risk of dementia. This association was confined to follow-up up to 4 years but not to follow-up after 4 years, suggesting either a role for reverse causality or only a short term effect of late-life physical activity in an elderly population.

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Chapter 4.2

Anxiety is not associated with the risk of dementia or cognitive decline

Renée F.A.G. de Bruijn, Nese Direk, Saira Saeed Mirza, Albert Hofman, Peter J. Koudstaal, Henning W. Tiemeier, M. Arfan Ikram

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Abstract

Background: Anxiety and depression frequently co-occur in the elderly and in patients with dementia. Prior research has shown that depression is related to the risk of dementia, but the effect of anxiety on dementia remains unclear. We studied whether anxiety symptoms and anxiety disorders are associated with the risk of dementia and cognition.
Methods: This study was embedded the prospective, population-based Rotterdam Study. In 1993-1995, anxiety symptoms were assessed in 2,708 non-demented participants using the HADS. In 2002-2004, anxiety disorders were assessed in 3,069 non-demented participants using the DSM-IV. In both study samples, participants were continuously monitored for dementia until January 1, 2011. Cognition was tested in 2002-2004 and at a follow-up visit in 2009-2011 in sample II only.
Results: In sample I, 358 people developed dementia and in sample II, 248 people developed dementia. We did not find an association with the risk of dementia for anxiety symptoms (HR 1.05, 95% CI 0.77;1.43) or for anxiety disorders (HR 0.92, 95% CI 0.58;1.45). We could demonstrate an association of anxiety disorders with poor cognition cross-sectionally, but this attenuated after additional adjustments.
Conclusions: Our findings do not offer evidence for an association between anxiety symptoms or anxiety disorders with the risk of dementia or with cognition. This suggests that anxiety is not a risk factor nor a prodrome of dementia in an elderly, community-dwelling population.

Introduction

Psychiatric disorders, such as anxiety and depression, are common co-manifestations in dementia.[1-4] Not only are anxiety and depression often diagnosed in demented people, but anxiety and depression may be one of the presenting symptoms of dementia.[1] Studies demonstrating a longitudinal association between depression and incident dementia suggest that depression might be a risk factor of dementia or alternatively an early clinical marker of incipient dementia.[5,6] Since anxiety shares both symptoms and risk factors with depression[7], it is often thought that anxiety is also associated with the risk of dementia. Here too, the underlying hypothesis is based on either a shared etiology between anxiety and dementia, or on anxiety as early symptom of incipient and yet to be diagnosed dementia.[7-9]

Previous studies examining the association between anxiety and dementia or cognition remain inconclusive.[8-23] Methodological differences, such as assessment of anxiety or selection of study participants might explain inconsistencies. Moreover, in some studies reporting an association between anxiety and increased risk of dementia, the influence of depression on these associations remains unclear.[8,10]

In the prospective, population-based Rotterdam Study we studied the association of anxiety symptoms and anxiety disorders with the risk of incident dementia. To further explore the effect of anxiety, we also related anxiety disorders to cognition, both cross-sectionally and longitudinally, in people without dementia.

Methods

Setting

The Rotterdam Study is a prospective, population-based cohort that started in 1990 and is conducted among inhabitants, aged 55 years and older, of Ommoord, a district of Rotterdam, The Netherlands. Of the 10,215 invited inhabitants, 7,983 (78%) agreed to participate in the baseline examination. Up until 2013, there have been 5 examination rounds. Details of the study have been described elsewhere.[24] For this study, two baselines were chosen because information on anxiety symptoms and anxiety disorders was collected at different examination rounds.

Anxiety symptoms were assessed in 1993-1995 and this examination was constituted as baseline for sample I. Of the 6,315 subjects that participated at baseline, a random half (N=3,060) was invited to undergo screening for anxiety symptoms using the Hospital Anxiety and Depression Scale (HADS). Of these, 83 participants were excluded because they were not sufficiently screened for anxiety symptoms. Furthermore, 44 people were excluded because they had prevalent dementia, 215 because they did not agree to undergo screening for dementia,

and 10 participants were excluded due to lack of follow-up data. Eventually, sample I comprised 2,708 non-demented subjects that underwent both screening for anxiety symptoms and had follow-up information on dementia diagnosis.
Anxiety disorders were assessed in 2002-2004 and this examination was constituted as baseline for sample II. Of the 3,550 participants that were eligible, a total of 3,259 people underwent screening for anxiety disorders. Of this sample, 124 people were excluded because they were prevalent demented, 56 because they did not agree to undergo screening for dementia, and 10 participants were excluded for lack of follow-up data. Finally, sample II comprised 3,069 non-demented subjects that underwent screening for anxiety disorders and had follow-up information on dementia diagnosis. Of the 3,069 subjects that were included in sample II, 1,506 subjects were also included in sample I. Follow-up for dementia for both samples was complete until January 1, 2011 (for 98.3% of potential person-years in sample I and for 96.9% of potential person-years in sample II). Sample II was also used to assess the association between anxiety disorders and cognition. Cognition was assessed at baseline in 2002-2004 and at follow-up in 2009-2011. Of the 3,069 subjects in sample II, 2,351 subjects had information on cognitive performance cross-sectionally and 1,115 subjects had information on cognitive decline longitudinally.
The medical ethics committee at Erasmus University of Rotterdam approved the study and written informed consent was obtained from all subjects.

Anxiety symptoms

Anxiety symptoms were assessed in 1993-1995 in a random half of the study population using the HADS.[25] The HADS is a brief questionnaire that is used often in the Netherlands for the assessment of anxiety symptoms and depressive symptoms. It consists of two subscales, the HADS-Depression (HADS-D) and HADS-Anxiety (HADS-A), each including 7-items. Total scores range from 0-21 with higher scores indicating more symptoms of depression or anxiety. A score of 8 or higher on the HADS-A scale was considered positive for anxiety symptoms.

Anxiety disorders

Anxiety disorders were diagnosed in 2002-2004 using a slightly adapted version of the Munich version of the Composite International Diagnostic Interview (M-CIDI).[26] We assessed the 12-month prevalence of anxiety disorders. The following anxiety disorders were assessed with a computerized diagnostic algorithm according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria: generalized anxiety disorder, panic disorder, agoraphobia, social phobia, and specific phobia.[27] These results were converted into a binary variable that stated whether a participant had at least one of the above-mentioned anxiety disorders or was free of any anxiety disorders. This variable was used in the analysis.

Assessment of dementia

Participants were screened for dementia at every examination round using a three-step protocol. Screening was done using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level.[28,29] Screen-positives (MMSE <26 or GMS organic level >0) subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[30] Participants who were suspected of having dementia, underwent, if necessary, further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. When information on neuro-imaging was required and available, it was used for decision making on the diagnosis. In the end, a consensus panel, led by a neurologist, decided on the final diagnosis in accordance with standard criteria for dementia (Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R)) and Alzheimer Disease (National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA))[31,32] using all available information. Follow-up for incident dementia was virtually complete until January 1, 2011.

Cognition

In sample II, cognitive performance was assessed at baseline in 2002-2004 and at follow-up in 2009-2011 with a cognitive test battery comprising Letter-Digit Substitution Task (LDST)[33], Stroop test[34], Verbal Fluency Test (VFT)[35], and 15-Word verbal Learning Test based on Rey's recall of words (15-WLT).[36] These tests tap into several cognitive domains: executive function, information processing, and memory function.[37] Higher test scores indicate better cognitive performance in all tests, except for the Stroop test, in which lower test scores indicate better cognitive performance. To calculate cognitive decline, we subtracted the test scores at the baseline examination from the test scores at the follow-up examination.

Other measurements

In sample I, depressive symptoms were assessed at baseline using the HADS-D subscale of the HADS.[25] A score of 9 or higher on the HADS-D scale was considered positive for depressive symptoms.[38] In sample II, depressive disorders were assessed at baseline in two steps. First, all participants were screened for depressive symptoms using the Center for Epidemiological Studies-Depression scale (CES-D).[39] Second, the participants with clinically significant depressive symptoms (CES-D ≥16) were invited for a semi-structured interview using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN).[40] Subsequently, depression

was defined according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR).[41] The category of clinical depressive disorders included the DSM-IV-TR-defined major depressive disorder, dysthymia, and depressive disorder not otherwise specified (including the former category of minor depression). Low educational level was defined as less than 12 years of education. Information on *apolipoprotein E (APOE)* genotype was obtained using polymerase chain reaction on coded DNA samples without knowledge of the dementia diagnosis.[42] Missing values in covariates (for every variable less than 6.5%) were imputed based on age and sex.

Statistical analyses

We used Cox proportional hazards models to assess the associations between anxiety symptoms and anxiety disorders with incident dementia. We also investigated the relation between the various subtypes of anxiety disorders and incident dementia. Plotting the Kaplan Meier curves did not reveal any overt violations of the proportional hazards assumption (Supplementary figures 1 and 2). The underlying time-scale in these models was the follow-up time. Follow-up time for both analyses was defined from time at assessment of anxiety symptoms or anxiety disorders until January 1, 2011. Subjects were censored within this time period when they were diagnosed with dementia, died, or decided to terminate their participation in the study. All models were adjusted for age and sex (basic model). In sample I, we adjusted subsequently for low educational level, *APOE*-ε4 carrier status, and depressive symptoms (fully adjusted model) for being potential confounders. In sample II we adjusted for the same potential confounders but instead of depressive symptoms now adjusted for depressive disorders. The same set of analyses was repeated for Alzheimer disease separately. To compare the results of samples I and II, we conducted a sensitivity analysis stratifying the follow-up time of sample I. A cut-off of 5.8 years was chosen because this was the mean follow-up time of sample II. We used linear regression models to examine the effect of anxiety disorders on cognition. These models were adjusted for age and sex (basic model), and subsequently for low educational level, *APOE*-ε4 carrier status, and depressive disorders (fully adjusted model). In the analyses with cognitive decline, all models were additionally adjusted for time between the baseline and follow-up assessment of cognition.
All analyses were performed using IBM SPSS statistics for Windows, Version 20.0 (IBM Corp, Armonk, NY).

Results

The baseline characteristics of our study samples are presented in Table 1. In sample I, 361 participants had anxiety symptoms. The mean follow-up in sample I was 11.8 years (stan-

Table 1. Population characteristics

	Sample I	Sample II
N	2,708	3,069
Age, mean (SD), years	68.6 (8.5)	75.5 (6.2)
Female, number (%)	1,495 (55.2)	1,810 (59.0)
APOE-ε4 carrier, number (%)	712 (28.1)	786 (26.8)
Low educational level, number (%)	797 (29.4)	850 (27.7)
Anxiety symptoms, number (%)	361 (13.3)	NA
Anxiety disorders, number (%)	NA	258 (8.4)
Depressive symptoms, number (%)	236 (8.7)	NA
Depressive disorders, number (%)	NA	81 (2.6)

Data are presented as means (standard deviations) or numbers (percentages). Percentages are calculated without missing data. For all reported variables, missing numbers occurred in 6.5% or less of all participants.
Abbreviations: N=number of people in sample; SD=standard deviation; *APOE=apolipoprotein E*; NA=not applicable.

dard deviation (SD) 5.0, total follow-up 32,047 person-years) during which 358 people were diagnosed with incident dementia, of whom 291 with incident Alzheimer disease. In sample II, 258 participants had anxiety disorders. Of these, 9 people had panic disorder, 51 specific phobia, 27 social phobia, 80 generalized anxiety disorder, and 127 people suffered from agoraphobia. The mean follow-up in sample II was 5.8 years (SD 1.9, total follow-up 17,778 person-years) during which 248 people were diagnosed with incident dementia, of whom 207 with incident Alzheimer disease.

We did not find an association between anxiety symptoms and incident dementia (Table 2). The HADS-anxiety and HADS-depression subscales were moderately correlated within our population (Spearman's rank correlation coefficient 0.62, p-value <0.001). However, addi-

Table 2. Associations between anxiety and incident dementia

	Dementia		Alzheimer disease	
	n/N	Hazard Ratio (95% CI)	n/N	Hazard Ratio (95% CI)
Anxiety symptoms				
Basic model	358/2,708	1.05 (0.77;1.43)	291/2,708	1.01 (0.71;1.43)
Fully adjusted model	358/2,708	0.99 (0.69;1.41)	291/2,708	1.07 (0.73;1.59)
Anxiety disorders				
Basic model	248/3,069	0.92 (0.58;1.45)	207/3,069	0.98 (0.60;1.59)
Fully adjusted model	248/3,069	0.81 (0.50;1.30)	207/3,069	0.87 (0.53;1.45)

Basic model: adjusted for age and sex
Fully adjusted model: adjusted for age, sex, low educational level, *apolipoprotein E*-ε4 carrier status, and depressive symptoms in anxiety symptoms or depressive disorders in anxiety disorders
Abbreviations: n=number of cases; N=number of people at risk; CI=confidence interval.

Table 3. Associations between anxiety symptoms and incident dementia, with stratified follow-up time

	Dementia		Alzheimer disease	
	Follow-up ≤ 5.8 years n/N=103/2,708 HR (95% CI)	Follow-up > 5.8 years n/N=255/2,243 HR (95% CI)	Follow-up ≤ 5.8 years n/N=80/2,708 HR (95% CI)	Follow-up > 5.8 years n/N=211/2,243 HR (95% CI)
Basic model	1.08 (0.63;1.85)	1.02 (0.70;1.50)	1.02 (0.55;1.89)	1.00 (0.66; 1.52)
Fully adjusted model	0.95 (0.52;1.74)	1.00 (0.64;1.55)	1.01 (0.51;1.99)	1.11 (0.69; 1.79)

Basic model: adjusted for age and sex

Fully adjusted model: adjusted for age, sex, low educational level, *apolipoprotein E*-ε4 carrier status, and depressive symptoms

Abbreviations: n=number of cases; N=number of people at risk; HR=hazard ratio; CI=confidence interval

tional adjustments for depressive symptoms and other potential confounders only slightly altered the associations.

People with anxiety disorders did not have an increased risk of dementia (Table 2). Consistent with these findings, no associations were found between subtypes of anxiety disorders and incident dementia. The corresponding hazard ratios (HR) were: generalized anxiety disorder (HR 0.46, 95% confidence interval (CI) 0.15;1.44), agoraphobia (HR 0.81, 95% CI 0.41;1.58), and specific phobia (HR 1.20, 95% CI 0.50;2.92). Associations remained stable after additional adjustments. Unfortunately, small sample size prevented us to investigate the relation between panic disorder or social phobia and dementia.

Results were similar for Alzheimer disease (Table 2) and after stratification of follow-up time (Table 3).

Participants with anxiety disorders performed poorer at baseline on the LDST (difference in cognitive performance -1.21, 95% CI -2.14;-0.29) and 15-WLT delayed recall (difference in cognitive performance -0.43, 95% CI -0.83;-0.04). These associations attenuated after adjusting for low educational level, *APOE*-ε4 carrier status, and depressive disorders (Table 4). We did not observe any associations between anxiety disorders and cognitive decline, except for the interference subtask of the Stroop test (fully adjusted difference in cognitive decline 5.05, 95% CI 0.98;9.11). However, the p-value of this association (p-value 0.02) would not have survived correction for multiple testing for seven cognitive tests. Additional information on mean cognitive test scores and mean cognitive decline is provided as supplementary material (Supplementary table 1).

Table 4. Anxiety disorders and cognition

	LDST (correct answers)	Stroop 1 (seconds)	Stroop 2 (seconds)	Stroop 3 (seconds)	VFT (animal names)	Immediate recall (correct answers)	Delayed recall (correct answers)
Cross-sectional (N=2,351)							
Basic model	-1.21 (-2.14; -0.29)	0.41 (-0.20; 1.02)	0.53 (-0.20; 1.25)	1.76 (-1.31; 4.84)	-0.73 (-1.47; 0.01)	-0.83 (-1.67; 0.01)	-0.43 (-0.83; -0.04)
Fully adjusted model	-0.66 (-1.57; 0.25)	0.37 (-0.24; 0.99)	0.43 (-0.30; 1.17)	0.97 (-2.14; 4.09)	-0.57 (-1.32; 0.17)	-0.44 (-1.28; 0.40)	-0.30 (-0.69; 0.10)
Longitudinal (N=1,115)							
Basic model	-0.17 (-1.16; 0.83)	0.48 (-0.29; 1.25)	0.73 (-0.04; 1.50)	4.74 (0.73; 8.76)	0.26 (-0.76; 1.29)	-0.28 (-1.47; 0.91)	0.00 (-0.53; 0.53)
Fully adjusted model	-0.27 (-1.27; 0.74)	0.45 (-0.34; 1.23)	0.74 (-0.05; 1.52)	5.05 (0.98; 9.11)	0.33 (-0.71; 1.37)	-0.36 (-1.56; 0.85)	-0.05 (-0.59; 0.49)

Values are the differences in cognitive performance (95% confidence interval) between people with anxiety disorders and those without anxiety disorders. For cross-sectional analyses the values are differences in test scores. For longitudinal analyses, the values are differences in change of test scores between the between baseline and follow-up assessment of cognition. Higher test scores indicate better cognitive performance in all tests, except for the Stroop test.

Basic model: adjusted for age, sex, and time between measurements (if applicable).

Fully adjusted model: adjusted for age, sex, low educational level, *apolipoprotein E* -ε4 carrier status, depressive disorders, and time between measurements (if applicable).

Abbreviations: LDST=Letter-Digit Substitution Task; Stroop 1=reading subtask of Stroop test; Stroop 2=color naming subtask of Stroop test; Stroop 3=interference subtask of Stroop test; VFT=Verbal Fluency Test; Immediate recall=15-Word Learning Test immediate recall; Delayed recall=15-Word Learning Test delayed recall; N=number of people included in analysis.

Discussion

In this study, we found that people with anxiety symptoms or anxiety disorders did not have an increased risk of dementia. People with anxiety disorders performed poorer in several cognitive tests at baseline, but these associations attenuated after additional adjustments. Moreover, we did not find an association between anxiety disorders and cognitive decline.

Strengths of this study are its prospective, population-based design and nearly complete dementia case finding. We thoroughly examined the association between anxiety and dementia using both anxiety symptoms and anxiety disorders. Furthermore, we assessed the association between anxiety disorders with cognitive performance cross-sectionally and cognitive decline longitudinally. This study also has limitations. Since we used two samples with different baselines, the subjects of sample I were younger than those of sample II, which may limit direct comparison of results. Moreover, there might be some survivor effect, because only the more healthy subjects survived up until the baseline of sample II. This effect was especially present when we examined the association between anxiety disorders and cognition, as a relatively small sample underwent the complete cognitive test battery. Unfortunately, we did not have information on duration of anxiety disorders and were not able to study the importance of duration of anxiety disorders on the risk of dementia. Furthermore, small sample size prevented us from examining the associations between some of the subtypes of anxiety disorders and dementia, which would be an interesting topic for further research. Finally, we studied a relatively homogeneous sample mainly of white, middle class people. This limits generalizability of our results to other populations.

The results of this study do not provide evidence for an association between anxiety symptoms or anxiety disorders and incident dementia. This suggests that anxiety is not a risk factor of dementia. Given that anxiety often occurs in dementia, there is a possibility that anxiety presents as a very early symptom, or prodrome, of dementia. However, when we stratified follow-up time in the association between anxiety symptoms and dementia, results remained similar. An explanation for our findings could be that anxiety occurs in a later phase of the dementia syndrome as a reaction to declining cognitive abilities.
As for cognition, we only found marginal effects of anxiety disorders on cognitive performance at baseline, which attenuated after additional adjustments. For longitudinal cognitive performance, we found an association between anxiety disorders and decline in performance on the interference subtask of the Stroop test. This effect estimate was also relatively small (only one third of the SD of the study sample, Supplementary table 1) and the association was not statistically significant after correction for multiple testing. However, it is possible that

anxiety disorders are associated with cognitive decline on a specific domain, such as executive function.[43-45] Further studies are needed to clarify this association.

Several studies have reported on the association between anxiety and dementia or cognitive decline, but results have been inconclusive.[8-23] There are various explanations for these inconsistent findings. Firstly, there was a large variation across studies regarding the assessment of anxiety; different questionnaires were used and anxiety was defined in various ways. Although we found similar results when we examined the association between anxiety symptoms and anxiety disorders with dementia, it is likely that some discrepancies between studies are due to methodological variability.

Secondly, there was also variability in selection of study participants. Whereas our study sample was a community-dwelling population, several other studies selected participants with mild cognitive impairment (MCI). One study found that among subjects with MCI, anxiety was a predictor for conversion to dementia, while for cognitively healthy subjects it was not.[13] Since MCI is an intermediate phase between normal aging and dementia, there is a possibility that anxiety appears as a presenting symptom in subjects, who are already on the verge of developing dementia. It is conceivable that people become more anxious as they notice a decline in cognitive performance and everyday functioning. Support for this hypothesis comes from a study that found among MCI patients anxiety symptoms increased with increasing cognitive and functional impairment according to the Clinical Dementia Rating score (CDR).[9] Another study found that MCI patients with anxiety had more abnormal cerebrospinal fluid (CSF) concentrations of amyloid-β42 and tau compared to MCI patients without anxiety symptoms.[46] These results could imply a shared etiology between anxiety and dementia. Although there was no difference in cognitive performance at baseline between the two groups of MCI subjects, this was a cross-sectional study and unable to detect cognitive decline. Therefore, it is possible that the MCI subjects with abnormal CSF concentrations were on the verge of developing dementia and were anxious because they had been noticing a decline in their cognitive performance.

Thirdly, when selecting participants from a memory clinic, there might be referral bias. For instance, people with an anxious personality are often more worried and might be more prone to visit a memory clinic when they notice only the slightest memory complaint. These people may be less likely to convert to dementia compared to non-anxious people who might visit a memory clinic only after experiencing more advanced memory complaints.[17] This could explain why some studies found a protective effect of anxiety on the risk of dementia in participants with MCI.[16,17]

Finally, an important consideration when examining the effect of anxiety on dementia is the overlap with depression.[7] One study, that assessed the association between several facets of neuroticism and dementia, observed an association between anxiety and increased risk of

dementia.[10] However, there was a moderate correlation with depression (correlation=0.68) for which models were not adjusted. Another large population-based study also found anxiety symptoms to be associated with dementia and Cognitive Impairment No Dementia (CIND) over a follow-up of 17 years.[8] However, models were only adjusted for psychological distress, and not specifically for depressive symptoms or depressive disorder. In both studies it therefore remained unclear to what extent the associations were independent of depression.

In conclusion, our data do not offer evidence for an association between anxiety symptoms or anxiety disorders and dementia. People with anxiety disorders had a worse cognitive performance at baseline, but these associations attenuated after additional adjustments. Moreover, anxiety disorders were not related to cognitive decline. These results suggest that anxiety is not a risk factor nor a prodrome of dementia in an elderly, community-dwelling population.

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Supplementary table and figures

Supplementary table 1. Cross-sectional test scores and longitudinal decline in test scores

	Cross-sectional test scores	Longitudinal decline in test scores
N	2,351	1,115
LDST, correct answers, mean (SD)	25.93 (6.70)	-2.82 (4.41)
Stroop 1, seconds, mean (SD)	19.00 (4.29)	0.49 (3.42)
Stroop 2, seconds, mean (SD)	25.07 (5.09)	1.60 (3.43)
Stroop 3, seconds, mean (SD)	59.41 (22.45)	7.00 (17.91)
VFT, animal names, mean (SD)	20.60 (5.15)	-1.46 (4.56)
Immediate recall, correct answers, mean (SD)	20.29 (5.89)	-1.15 (5.26)
Delayed recall, correct answers, mean (SD)	6.43 (2.71)	-0.55 (2.35)

Data represent means (standard deviations). Decline in test scores was the change of test scores between the between baseline and follow-up assessment of cognition. Higher test scores indicate better cognitive performance in all tests, except for the Stroop test.
Abbreviations: N=number of people; LDST=Letter-Digit Substitution Task; SD=standard deviation; Stroop 1=reading subtask of Stroop test; Stroop 2=color naming subtask of Stroop test; Stroop 3=interference subtask of Stroop test; VFT=Verbal Fluency Test; Immediate recall=15-Word Learning Test immediate recall; Delayed recall=15-Word Learning Test delayed recall.

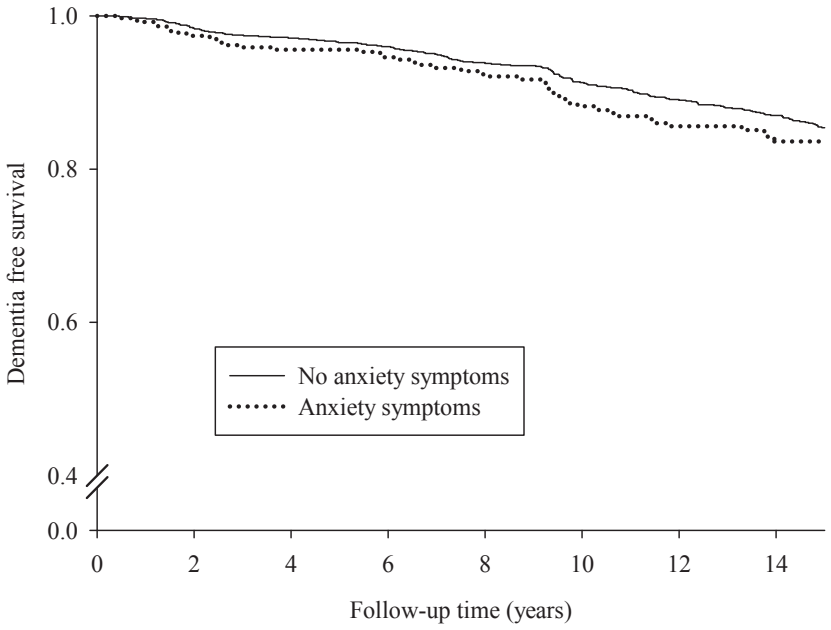

Figure 1. Kaplan-Meier curve of dementia free survival of anxiety symptoms versus no anxiety symptoms

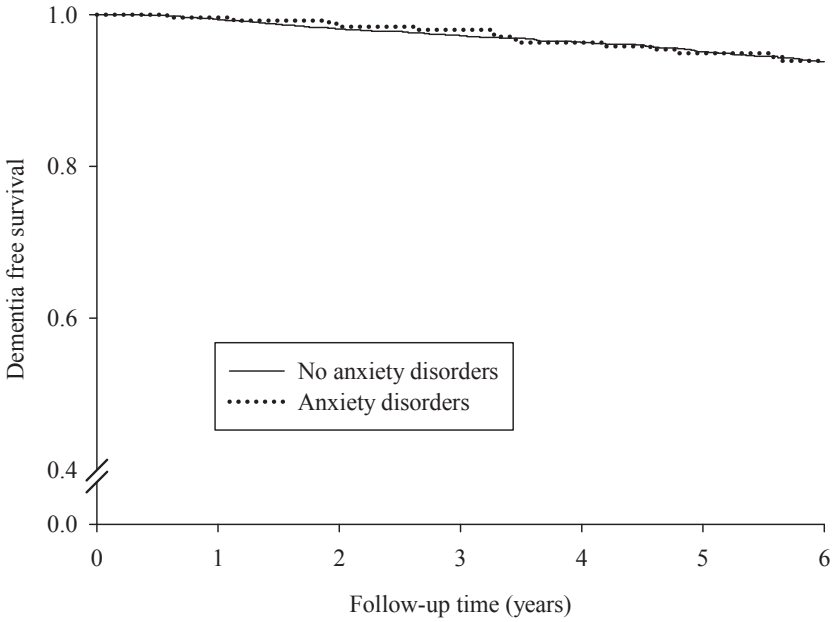

Figure 2. Kaplan-Meier curve of dementia free survival of anxiety disorders versus no anxiety disorders

Chapter 4.3

Depressive symptoms predict incident dementia during short- but not long-term follow-up period

Saira Saeed Mirza, Renée F.A.G. de Bruijn, Nese Direk, Albert Hofman, Peter J. Koudstaal, M. Arfan Ikram, Henning W. Tiemeier

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Abstract

Background: Whether depression is a long term risk factor of dementia or represents a dementia prodrome is unclear. Therefore, we examined the relationship between depressive symptoms and dementia both during short and long follow-up in a population-based cohort.
Methods: In the Rotterdam Study 4,393 non-demented individuals were followed for incident dementia for 13.7 years by continuous monitoring. Cox proportional-hazards models for different time intervals were used to estimate the risk of incident dementia.
Results: 582 participants developed dementia during 13.7 years. Persons with depressive symptoms had an 8% increased risk of dementia compared to those without depressive symptoms during the over-all follow up. The risk was highest in the short and intermediate follow-up particularly in men. We did not find an association in the follow-up period beyond 10 years.
Conclusion: Our results suggest that late-life depressive symptoms are part of a dementia prodrome rather than an independent risk factor of dementia.

Introduction

Dementia poses a high burden on society and health care, both in terms of financial costs as well as suffering for patients and care-givers. Current estimates indicate a prevalence of 35.6 million patients worldwide with another 7.7 million incident cases occurring annually.[1] In order to develop effective preventive and therapeutic strategies, it is crucial to unravel the multi-factorial etiology of dementia.

Depression and depressive symptoms are very common in the elderly and often co-occur with dementia.[2] Depression and dementia share many vascular risk factors[3] and various studies have shown that depression in late life is associated with a 2 to 5 fold increased risk of dementia.[4-8] Most studies investigated this association over a follow-up period of at most 7 years. In contrast, the Framingham Study studied a follow-up period of 17 years and reported a 70% greater risk of incident dementia in depressed individuals; however, the investigators did not distinguish the risk between short and long-term follow-up. Taken together, current data suggest a strong association between depression and incident dementia, but the question remains whether depression is a risk factor of dementia or merely a prodromal symptom of underlying dementia.[9] Given the long pre-clinical phase of dementia, it is conceivable that subclinical dementia causes depressive symptoms rather than depression being a true risk factor of dementia. One way to address this issue is to study the association of depression and dementia during a long follow-up and then explore the association over separate incremental periods of follow-up. The hypothesis to be tested is that there is a strong association between depression and dementia over a short follow-up period which attenuates with longer follow-up.

Also, some studies have suggested a difference between men and women in the association of depression with dementia, but data are still scarce.

Therefore, we studied the relationship of depressive symptoms and dementia both over long and short follow-up periods in a population based cohort. We further examined if the relationship between depression and dementia differs between men and women.

Methods

Setting

This study was embedded in the Rotterdam Study; an ongoing population-based prospective study of the elderly that started 1990 and studies the incidence and determinants of chronic diseases in late life.[10] The Medical Ethics Committee of Erasmus Medical Center Rotterdam approved the study and a written informed consent was obtained from all participants.

Every 3 to 4 years, all participants undergo an extensive home interview and a physical ex-

amination at the research center. In addition, all participants are continuously monitored for the occurrence of all major events during follow-up by linkage of the study database with medical files from general practitioners. The third examination round of the Rotterdam study constituted the baseline of this study, because the data on depressive symptoms were complete and uniformly collected (using CES-D for the whole cohort).

Study population

Of the original cohort of 7,983 persons in 1990, 4,797 surviving persons participated at the third examination which took place from 1997-1999. From these 4,797 individuals, 4,602 (96%) completed the depression assessment questionnaire. Of these 4,602, 110 participants did not consent to undergo dementia screening and were excluded. We also excluded 92 participants who were demented at baseline and 7 who were lost to follow up. This yielded a total of 4,393 individuals (92% of total survivors) available for final analysis, who were followed for a maximum of 13.7 years (mean 8.7, SD 3.5 years) for incident dementia. Follow-up started from the day of depressive symptom screening to the date of incident dementia, date of death, or the censor date January 1st, 2011, whichever occurred first.

Assessment of depression

We used the validated Dutch version of the Center for Epidemiology Depression Scale (CES-D) for assessment of depressive symptoms at baseline. The CES-D comprises of 20 questions, each with a possible score of 0-3, and the score indicates clinically relevant depressive symptoms. Depressive symptom scores were used as a standardized continuous variable. Z scores were calculated as weighted individual score minus mean score, divided by the standard deviation. CES-D scores were weighted by missing values only if missing values did not exceed 25%. For descriptive purposes however, a score of 16 or higher is considered suggestive of depressive symptoms.[11]

Assessment of incident dementia

Participants were screened for dementia at baseline and follow-up examinations using a three-step protocol.[12] Screening was done using the Mini-Mental State Examination (MMSE)[13] and the Geriatric Mental Schedule (GMS) organic level.[14] Screen-positives (MMSE<26 or GMS organic level>0), subsequently underwent an examination and informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).[15] Participants who were suspected of having dementia, underwent, if necessary, further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and digitized medical records from general

practitioners and the Regional Institute for Outpatient Mental Health Care. In the end, a consensus panel, led by a neurologist, decided on the final diagnosis in accordance with standard criteria using the DSM-III-R criteria for dementia and the NINCDS-ADRDA for Alzheimer disease.[16] If required for differential diagnosis, neuro-imaging was used. Follow-up for incident dementia was virtually complete (98.6%) till January 1st, 2011.

Covariates

In addition to age and gender, education level, smoking, cognition level at baseline, hypertension, diabetes mellitus, prevalent stroke, and use of antidepressant medication were considered possible confounders. Smoking, hypertension, diabetes and stroke are well documented risk factors for all types of dementia. A low education level has been found to be associated with increased risk of dementia, especially in females.[17] In subsequent models, we adjusted for marital status, *apolipoprotein* (*APOE)* ε4 carrier status, cognitive complains at baseline, and psychotropic medication use.

Education level was assessed during the interview and people were classified into two categories; low level of education (primary only or primary and unfinished secondary) and intermediate to high (primary and secondary, vocational or university). Cognition was assessed by the MMSE at baseline.[13] Inquiring about smoking habits, participants were categorized into current, former and never smokers. Blood pressure was measured twice at the right arm in sitting position at the research center; average of two blood pressure readings was used. Hypertension was defined as systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg, or use of antihypertensive medication assessed by interview and pharmacy records.[18] Diabetes Mellitus type II was diagnosed as fasting blood glucose ≥ 126.13 mg/dl (multiply by factor 0.0555 to covert to mmol/l) or use of anti-diabetic medication evaluated by interview and pharmacy records.[19] Previous stroke was determined by reported events on interview and confirmed by medical records. In addition, participants are continuously monitored for all major events through automated linkage of study database with GP files.[20] Information on use of antidepressants (ATC code n06) was obtained by interview, medical and pharmacy records.

Statistical analysis

We used Cox proportional hazards model to assess the relationship between clinically relevant depressive symptoms and incident dementia (all cause). We investigated not only the total follow-up period as a whole, but also separate 5-year time periods (0-5 years, 5-10 years and more than 10 years of follow up). This analysis was performed to study the timing of incident dementia in relation to the appearance of depressive symptoms. However several studies have used a follow-up time range of 1 to 5 years to study the short-term effect of

depression on dementia incidence[5,6,21-24]; there is no recommended cut-off of follow-up time to study depression as a dementia prodrome. In an alternative analysis, time periods were defined by ensuring equal number of 100 cases in each period (five periods 100 incident cases, the last period only counted 82 cases). This approach allows a more detailed assessment of risk ratio change over time and increases the power to detect changes. Hazard ratios of dementia for each time period of 100 cases were calculated separately as well as cumulatively. The cumulative time approach in both the main analysis and in the 100 case-analysis was a method carried out to ensure comparability with other studies, as most studies have examined the association of depressive symptoms and dementia using a Cox model and with using variable follow-up periods. Therefore, we first used a cumulative follow-up approach in which we examined the association between depression and dementia by increasing the years of follow-up by not changing the baseline (i.e. 0-5 years, 0-10 years, and 0-13.7 years). Similarly, for the 100-case analysis, we examined association of depression and dementia by increasing 100 cases in every subsequent step without changing our baseline (i.e. baseline-100 cases, baseline-200 cases, baseline to 300 cases and so on).

As secondary analyses, we explored effect modification by gender and age (median used as cut-off) using stratification and interaction terms. Additionally, we analyzed our data, using only Alzheimer disease as outcome.

We ran an additional analysis to test sensitivity of our findings. Analysis was repeated excluding persons with clinically relevant depressive symptoms occurring prior to baseline. Depressive symptoms were also assessed, 4 years prior to baseline either with the CES-D (cut-off ≥ 16) in 48% of participants, or the Hospital Anxiety and Depression Scale, HADS (cut-off ≥ 9) in 52% of participants, as part of a pilot.[25] In this sensitivity analysis, the effect of more chronic depressive symptoms, which are less likely to be an indicator of dementia prodrome, is reduced. If there were an effect of depression as part of a dementia prodrome only, our hazard ratios for the short follow-up would be expected to increase slightly if chronic cases are excluded.

Since, depressive symptoms in the Rotterdam study were re-measured at a follow-up round in 2002-2004, we also repeated our analysis using depressive symptoms assessed at this follow-up round as our baseline.

Although we have used depression as a standardized continuous variable in all analyses, we also performed our main analysis using CES-D score dichotomized at 16 points.

Results are presented as hazard ratios (HR) with 95 % confidence intervals (CI). All analyses were adjusted for age and gender in the first model and additionally for education, smoking, hypertension, diabetes, prevalent stroke, MMSE score and antidepressants' use in the second model. Data were analyzed using the Stata Software Version 12 (StataCorp, College Station, TX, USA).

Results

Baseline characteristics of the study population are summarized in Table 1. The study included 4,393 individuals comprising 59% females (n=2,599). Mean age at baseline was 73 years (SD 7.3 yrs; range 61.1-105.8 yrs) and participants were followed for a maximum of 13.7 years (mean 8.7, SD 3.5 yrs). Applying the accepted cut-off of ≥16 for CES-D, seven percent (n=323) of the study population had clinically relevant depressive symptoms at baseline; however, we used continuous depression scores for all analyses.

Results using depressive symptoms as a dichotomized variable showed similar pattern and are shown in Supplementary table 2.

Of the 4,393 individuals, 13% (n=582) developed dementia (all cause); 84% of those were Alzheimer disease cases (n=489). Mean age of dementia diagnosis was 83 years (SD 6.3; range 65.5-102 yrs).

When investigating each time period with 100 subsequent cases separately, the risk of dementia decreased gradually from (HR 1.24, 95% CI 1.06;1.44) in the first period, to (HR 0.89, 95%

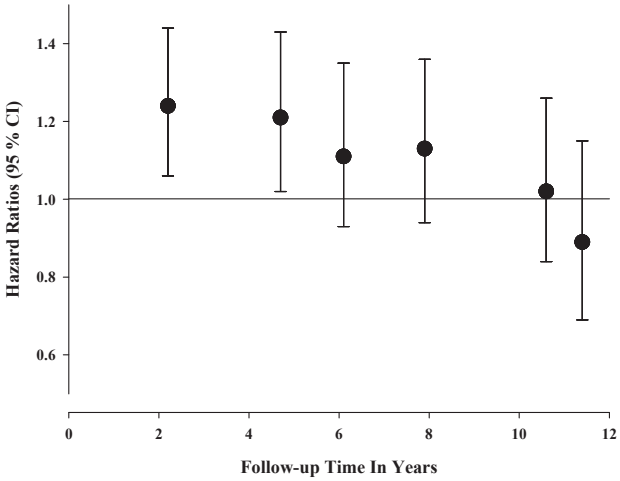
Table 1. Baseline characteristics of the study population, RS-I-3*-N=4,393

Characteristics	Descriptives	
Females, n(%)	2,599	(59.2)
Age, mean (SD)†yrs	72.7	(7.3)
BMI, mean (SD)	26.86	(3.7)
Education		
Low level of education	1,350	(31.2)
Intermediate to high	2,980	(68.8)
Smoking Status, n (%)		
Never	1,520	(34.6)
Former	2,157	(49.1)
Current	716	(16.3)
Diabetes, n (%)	534	(12.2)
Stroke, n (%)	338	(7.7)
Myocardial Infarction, n (%)	427	(9.7)
Hypertension, n (%)	2,990	(68.1)
Total cholesterol (mmol/l), mean (SD)	5.82	(0.9)
HDL-cholesterol (mmol/l), mean (SD)	1.39	(0.4)
Antipsychotic use, n (%)	642	(14.6)
Antidepressant use, n (%)	135	(3.1)
Clinically Relevant Depressive Symptoms, n (%)	323	(7.4)

* RS-I-3-Rotterdam Study-Cohort I-Examination round 3

† SD-Standard deviation

1a: Depressive symptoms and risk of incident dementia per follow-up interval



1b: Depressive symptoms and risk of incident dementia with cummulatively increasing follow-up

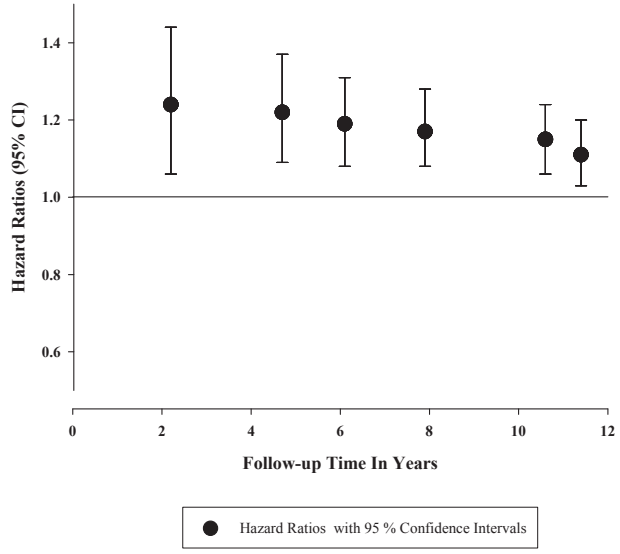


Figure 1: Depressive symptoms and risk of incident dementia-Effect estimates per 100 consecutive incident dementia cases

Adjusted hazard ratios for risk of dementia at different time points in depressed individuals (N=4393)
Total number of dementia cases (N=582) were split in subgroups of 100 patients according to incidence of dementia. Hazard ratios for these 100 cases were calculated. Mean follow-up time for each subgroup of 100 additional cases is used to plot the graphs.
The circles represent the hazard ratios and the lines represent the 95 % confidence intervals.

Table 2. Clinically relevant depressive symptoms and risk of incident dementia-overall and gender split analysis, N=4,393

	Follow-up time in years			
Depressive symptoms	**Overall follow-up**	**0-5 years**	**5-10 years**	**10-13.7 years**
Cases/N	582/4,393	222/4,393	238/3,529	122/2,554
	HR (95% CI)			
Depressive score, (per SD)†	1.11 (1.03;1.20)	1.19 (1.07;1.33)	1.15 (1.03;1.29)	0.84 (0.68;1.05)
Depressive score, (per SD)‡	1.08 (1.00;1.17)	1.13 (1.01;1.27)	1.14 (1.01;1.29)	0.83 (0.66;1.04)
Males (N=1794)				
Cases/N	176/1,794	62/1,794	71/1,416	43/985
Depressive score, (per SD)†	1.20 (1.02;1.41)	1.51 (1.23;1.86)	1.20 (0.91;1.58)	0.40 (0.17;0.93)
Depressive score, (per SD)‡	1.04 (0.88;1.24)	1.31 (1.04;1.66)	1.06 (0.79;1.41)	0.38 (0.16;0.93)
Females (N=2599)				
Cases/N	406/2,599	160/2,599	167/2,113	79/1,569
Depressive score, (per SD)†	1.09 (1.01;1.19)	1.12 (0.99;1.27)	1.14 (1.01;1.30)	0.94 (0.75;1.18)
Depressive score, (per SD)‡	1.09 (1.00;1.18)	1.10 (0.96;1.26)	1.15 (1.01;1.31)	0.92 (0.73;1.15)

Cases refer to incident dementia cases

† age and gender adjusted

‡ additionally adjusted for smoking, education, hypertension, diabetes, prevalent stroke, MMSE and anti-depressants' use

Depression is taken as a continuous standardized variable using the CES-D scores

CI 0.69;1.15) in the last period. Similar pattern was observed when we calculated hazards for cumulative time periods of 100 cases; (HR 1.24, 95% CI 1.06;1.44) in the first period to (HR 1.11, 95% CI 1.03;1.20) in the last period (Figure 1).

In the overall follow-up of 13.7 years, depressive symptoms were associated with a moderately increased risk of incident dementia (Table 2).

During a shorter follow-up time i.e. 5 years from baseline, depressive symptoms were associated with a high risk of incident dementia (fully adjusted HR 1.13, 95% CI 1.01;1.27). The same was true for the period of 5 to 10 years follow-up (HR 1.14, 95% CI 1.01;1.29). In contrast, we did not find any relationship in the third follow-up period in the fully adjusted model (HR 0.83, 95% CI 0.66;1.04). (Table 2)

After additional adjustments for marital status, *APOE*-ε4 carrier status, cognitive complaints at baseline and use of psychotropic drug use, our results remained unchanged.

In a secondary gender split analysis, we found a more pronounced effect in men than in women, though the overall pattern in both sexes was similar to the main analysis (Table 2). In depressed men however, the risk of dementia in the 10-13.7 year interval was reduced by 60% (HR 0.38, 95% CI 0.16;0.93). The interaction for gender was statistically significant ($p<0.001$). Interaction for age was not significant, hence not shown.

Repeating all analyses using Alzheimer disease as outcome yielded similar results and patterns (Supplementary table 1).
In the sensitivity analysis, after excluding individuals who had been screened positive for clinically relevant depressive symptoms 4 years prior to baseline, the presence of depressive symptoms showed a 16% higher risk of incident dementia in the 0-5 year follow-up (HR 1.16, 95% CI 1.02;1.32).
Using depressive symptoms assessed at the 2002-2004 examination round, we found a similar pattern of results as the main analysis (data not shown).

Discussion

We found that persons with depressive symptoms had a higher risk of incident dementia, including Alzheimer disease. These associations were strongest for short follow-up time and attenuated with incrementally longer follow-up periods. Furthermore, the association was more pronounced in men than in women.
Prevalence of depressive symptoms is relatively low in this cohort. However it falls within the variable range of 2.8% to 35% reported in a review of depressive symptoms in the elderly.[26] In addition, the SHARE study has reported the Netherlands to be one of the lower depression prevalence countries in Europe.[27]
Strengths of the study include the large population-based cohort followed for over 13 years. However, certain methodological considerations need to be mentioned. First, we included clinically relevant depressive symptoms (assessed by CES-D) as the determinant rather than diagnosed depression. Therefore, we cannot be certain about the generalizability of our results to clinical depressive syndromes as well. Second, some residual confounding due to unknown or unmeasured confounders such as physical activity and diet cannot be completely ruled out. Third, there is a possibility that some selection through depressive symptoms and/or cognitive function in the previous examination round may have influenced the results. Fourth, depressive symptoms were related to death of participants in the first ten years of follow-up, so there is a possibility of some selection by death in the 10-13.7 year interval.
There are a few possible explanations for our observation that the association of depression with incident dementia was strongest with short follow-up and attenuated with longer follow-up. First, late-onset depressive symptoms preceding dementia could be merely a reactive phenomenon. It is possible that depression is a psychological response to the ongoing cognitive decline.[28] Second, late onset depressive symptoms may represent a prodrome of dementia. The prodrome can be defined as a pre-dementia syndrome in which the underlying subclinical dementing process manifests itself by depression or altered behavior, thus marking the onset of clinical dementia in the near future. Where a depression in adult life or a lifetime

history of depression would be considered a risk factor for dementia, a very recent history of late-onset depressive symptoms may be an early clinical manifestation of the underlying neurodegenerative condition. This implies that both depression and dementia are the result of a common underlying process(es) but that symptoms of depression manifest earlier than dementia. It has been shown that patients experiencing cognitive decline together with late onset depression develop dementia within a few years after the onset of depression.[28] This prodromal hypothesis is in line with recent findings from large cohort studies suggesting that late-onset depression is a prodrome of dementia onset.[21,29] Studies have reported a positive association between depression and dementia over a shorter follow-up period of at most 5 years.[4-7] In a subset of the Rotterdam Study, we previously reported a null association between depressive symptoms and dementia; however, differences in sample size, follow-up, age and cognition at baseline could explain the difference in results.[30]

Third, depression may increase the risk of dementia over a short term period only. Some individuals with depressive symptoms may be more vulnerable for incident dementia because of certain genetic or environmental risk factors although a shared etiology would typically convey a constant risk over time.[3,31] Several potential biological mechanisms could be a common intermediate between depression and dementia such as hippocampal atrophy.[2] It has also been shown that depressed individuals have low levels of adrenaline[32] and serotonin[33], and deficits of these monoamines are also associated with increased severity of dementia.

The underlying neurodegenerative process, might get accelerated due to depression, by the activation of hippocampal pituitary axis leading to increased cortisol levels, hence precipitating dementia.[28]

We also found that the association of depressive symptoms and dementia in the short term period was stronger in men than in women. This finding concurs with two prospective cohort studies which reported a stronger association of depressive symptoms and risk of incident dementia in men compared with women.[34,35] There is limited literature available in this context, and further epidemiological and etiological studies are highly recommended to better understand the gender differences in association between depression and dementia.

We observed a protective association between depression and dementia in the 10-13.7 year interval in men. However, since this period has a fewer number of cases (n=43) the estimates may not be very precise. It is also possible, that reduced effect estimates are a result of other competing risks in this period and deaths occurring due to other causes such as cardiovascular causes or cancers. Additionally, we speculate that perhaps depressed persons who survive ten years or more, become resilient against subsequent dementia.

In conclusion, late-onset depressive symptoms represent a part of the prodromal stage of dementia, rather than being a risk factor for dementia. Depressive symptoms posed a much higher risk of incident dementia in men compared with women in a short follow-up.

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Supplementary tables

Supplementary table 1. Clinically relevant depressive symptoms and risk of Alzheimer disease, N=4,393

	Follow-up time in years			
Depressive symptoms	**Overall follow-up**	**0-5 years**	**5-10 years**	**10-13.7 years**
Cases/N	489/4,393	194/4,393	199/3,529	96/2,554
	HR (95% CI)			
Depressive score, (per SD)†	1.08 (0.99;1.17)	1.16 (1.03;1.31)	1.11 (0.98;1.27)	0.79 (0.61;1.03)
Depressive score, (per SD)‡	1.05 (0.96;1.15)	1.11 (0.98;1.26)	1.09 (0.95;1.25)	0.79 (0.60;1.04)
Males (N=1794)				
Cases/N	131/1,794	43/1,794	55/1,416	33/985
Depressive score, (per SD)†	1.16 (0.95;1.41)	1.38 (1.04;1.82)	1.30 (0.98;1.73)	0.43 (0.17;1.06)
Depressive score, (per SD)‡	1.02 (0.83;1.25)	1.16 (0.83;1.60)	1.13 (0.83;1.53)	0.48 (0.19;1.22)
Females (N=2599)				
Cases/N	358/2,599	151/2,599	144/2,113	63/1,569
Depressive score, (per SD)†	1.07 (0.97;1.17)	1.13 (0.99;1.28)	1.08 (0.93;1.24)	1.08 (0.93;1.24)
Depressive score, (per SD)‡	1.06 (0.96;1.16)	1.11 (0.97;1.27)	1.07 (0.92;1.25)	1.07 (0.92;1.25)

* Cases refer to incident Alzheimer disease cases

† Age and gender adjusted

‡ Additionally adjusted for education, smoking, hypertension, diabetes, prevalent stroke, MMSE score and antidepressants' use

§ Depression is taken as a continuous standardized variable using the CES-D scores

Supplementary table 2. Clinically relevant depressive symptoms and risk of incident dementia, N=4,393

	Follow-up time in years			
Depressive symptoms	**Overall follow-up**	**0-5 years**	**5-10 years**	**10-13.7 years**
Cases/N	582/4,393	222/4,393	238/3,529	122/2,554
	HR (95% CI)			
Depressive symptoms†	1.46 (1.13;1.89)	1.43 (0.96;2.15)	1.94 (1.33;2.82)	0.74 (0.34;1.59)
Depressive symptoms ‡	1.38 (1.06;1.80)	1.26 (0.83;1.91)	1.94 (1.31;2.87)	0.74 (0.34;1.60)

*Cases refer to incident dementia cases

† age and gender adjusted

‡ additionally adjusted for smoking, education, hypertension, diabetes, prevalent stroke, MMSE and anti-depressants' use

§ Depression is used as dichotomized (cut-off CES-D score ≥ 16 taken as positive for depressive symptoms)

Chapter 5

General discussion

The aim of this thesis was to assess the impact of known risk factors of dementia and to discover novel risk factors. In this chapter, I will first summarize the main findings of the studies. Next, I will discuss methodological considerations concerning the work and finally, clinical implications and directions for future research.

Main findings

Conventional cardiovascular risk factors

Alzheimer disease is by far the most common subtype of dementia. When Alois Alzheimer examined the brain of Auguste D., the first patient with Alzheimer disease, he found three striking neuropathological features: amyloid plaques, neurofibrillary tangles, and cerebrovascular pathology. Since then, amyloid plaques and neurofibrillary tangles are considered hallmarks of the disease[1], but the role of vascular pathology has been less well acknowledged. However, in the last decades, accumulating evidence has shown that cardiovascular diseases and risk factors too are important factors in Alzheimer disease.[2-4] When reviewing the literature, I found there is abundant evidence that stroke and heart diseases are related to an increased risk of Alzheimer disease (Chapter 2.1).[5-10] Similar to dementia pathology, cardiovascular pathology slowly accumulates over years before manifesting as clinical event. Preclinical markers of cardiovascular disease can be visualized with various imaging techniques. Several of these markers, such as intima media thickness of the carotid artery and white matter lesions and lacunar infarcts in the brain, have been related to Alzheimer disease.[7,11-13] Additionally, cardiovascular risk factors, such as diabetes mellitus and smoking, have also been associated with an increased risk of Alzheimer disease.[14,15] The relations of other cardiovascular risk factors, such as hypertension, hypercholesterolemia, and obesity with dementia are more complex and appear to differ with age.[16-18] These risk factors are associated with an increased risk of Alzheimer disease when assessed during midlife, whereas at late-life there is either no clear association or even an inverse association.[16-18] Cardiovascular diseases and risk factors, unlike dementia, can be treated. Therefore, the next step in dementia research is whether optimal treatment and prevention of these risk factors can prevent dementia. Previous studies have estimated this preventive potential for dementia, but results differed widely due to methodological variation. Some studies did not take into account the interaction between cardiovascular risk factors when estimating the combined population attributable risk (PAR). Furthermore, most studies used data from the nineties, whereas the incidence of dementia has declined since then, probably due to improved treatment and prevention of cardiovascular risk and improvement of educational level.[19-23] Therefore, previous values of PAR might have become overestimates. In Chapter 2.2, I estimated that about one quarter to one third of dementia cases could be prevented by optimal treatment of cardiovascular diseases, cardiovascular risk

factors, and improvement of educational level. Furthermore, I found that this potential for prevention has not declined over the last two decades, as it was 25% in the original cohort (followed from 1990-2000) and 33% in the extended cohort (followed from 2000-2010). I did find, however, that the relative importance of individual risk factors had changed over time. The effects of hypertension, diabetes, lower educational level, stroke, and coronary heart disease were larger in the extended cohort than in the original cohort. Conversely, the effects of smoking, unfavorable cholesterol levels, and atrial fibrillation had declined. Figure 1 shows the contribution of each risk factor to the PAR per cohort. The sum of the separate risk factors is somewhat higher than the combined PAR because the latter metric is adjusted for the interaction between risk factors. These findings provide contemporary information on preventive options for dementia and call for urgent public health interventions.

Dementia is diagnosed only after a long preclinical phase during which neuropathology and clinical symptoms gradually accumulate. Preventive options for dementia should therefore be targeted early, preferably years before dementia is diagnosed. Mild cognitive impairment (MCI) is the clinical correlate of the intermediate phase between normal aging and cognitive impairment of early dementia. MCI has been widely investigated, but findings across studies vary largely.[24] We found that about 10% of our population-based sample had MCI (Chapter 2.3). Several known risk factors of dementia, such as higher age, *APOE*-ε4 carrier status, and stroke were related to MCI when assessed at baseline, but also up to seven years prior to baseline. Some associations differed over time. Lower total cholesterol levels were related to MCI when assessed at baseline, but not when assessed before baseline. Current smoking and lower HDL-levels were related to MCI only when assessed before baseline. We also investigated MRI-correlates of dementia and found that participants with MCI, especially those with non-amnestic MCI, had larger white matter lesion volumes, a worse microstructural integrity of the normal-appearing white matter, and more lacunar infarcts than cognitively healthy participants. Furthermore, participants with MCI had an increased risk of dementia and mortality. Given that cardiovascular risk and MRI-correlates of cerebrovascular disease disease were related to early phases of cognitive decline, which in turn related to an increased risk of dementia and mortality, these results underline the importance of vascular factors even in the early phases of dementia.

Emerging cardiovascular and metabolic risk factors

As described above, several heart diseases have been associated with an increased risk of dementia.[8-10] Nevertheless, the role of atrial fibrillation in dementia is still controversial, as several studies did not find an association between these diseases.[25-30] Most of these studies included very old participants and it is possible that atrial fibrillation needs to start at a younger age to contribute to the etiology of dementia. Indeed, we found that atrial fibrillation

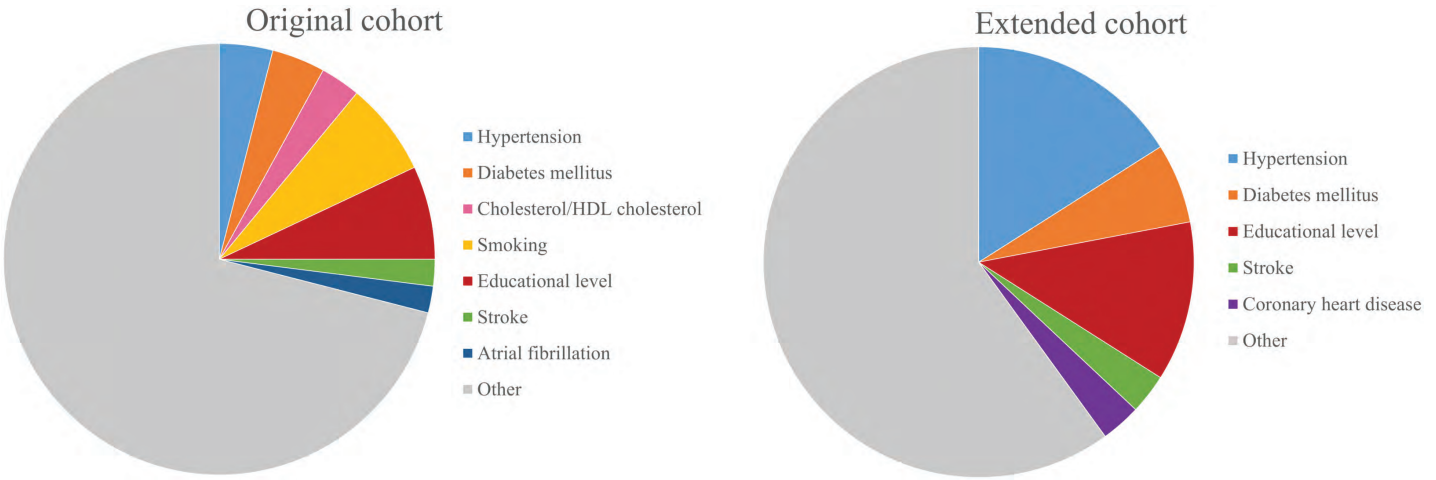

Figure 1. Contribution of each dementia risk factor to the population attributable risk

was related to an increased risk of dementia in the younger participants of our study population only (stratified at median age of 67 years) (Chapter 3.1). Atrial fibrillation could lead to dementia via hypoperfusion or microemboli.[31,32] A non-causal mechanism is shared etiology, since both diseases share many risk factors.[31,32] However, we found that the longer the younger participants suffered from atrial fibrillation, the higher their risk on dementia was. Hence, our results are more suggestive of a causal relation between atrial fibrillation and dementia.

Besides clinical heart diseases, further investigating the role of subclinical cardiovascular disease in dementia might provide novel options for prevention. Cardiac function is often impaired in the absence of clinical heart disease.[33] In Chapter 3.2, we investigated whether measures of subclinical cardiac dysfunction are related to stroke and dementia. Using echocardiography measures, we found that diastolic dysfunction was related to an increased risk of both stroke and dementia, whereas systolic dysfunction was related to an increased risk of stroke only. Diastolic dysfunction was also related to silent infarcts, especially lacunar infarcts. Several other studies also found that worse subclinical cardiac function is related to stroke and worse cognitive function cross-sectionally, but our study is one of the first to confirm this association longitudinally.[34,35] Atherosclerosis is another marker of subclinical cardiovascular disease. A reliable measure of atherosclerotic plaque burden is calcification volume of the plaque assessed by non-enhanced computed tomography.[36] Larger calcification volumes in the aortic arch, and extracranial and intracranial arteries were related to an increased risk of dementia. These associations were similar for Alzheimer disease and slightly attenuated after adjusting for cardiovascular risk factors and censoring for stroke. Calcification volumes in all vessel beds were associated with an increased risk of cognitive decline in non-demented people. These results suggest that generalized atherosclerosis, rather than localized atherosclerosis, is related to dementia. Since we also found an effect of atherosclerosis in the early phases of cognitive decline, reducing atherosclerosis might be another potential target for prevention of dementia (Chapter 3.3). A measure closely related to atherosclerosis is cerebral vasomotor reactivity, which reflects the ability of the cerebral arterioles to dilate in the event of hypercapnia to improve cerebral blood flow.[37,38] In Chapter 3.4, we found that a lower cerebral vasomotor reactivity was related to an increased risk of mortality, especially cardiovascular mortality. Lower vasomotor reactivity has previously been related to cognitive decline cross-sectionally, but future studies should unravel whether cerebral vasomotor reactivity is also longitudinally associated with an increased risk of dementia.[39]

Although cardiovascular risk is important in dementia, it is not the only causal factor. For example, metabolic factors, like insulin-like growth factor-I (IGF-I), have also been investigated in this matter. IGF-I has several neuroprotective features as it is involved in neurogenesis, reduction of amyloid β, and tau phosphorylation.[40-42] This suggests that low levels of IGF-I could increase the risk of dementia. However, previous studies have been inconclusive.[43,44] Research on IGF-I is hampered by the fact that most of the circulating IGF-I is bound to IGF-I

receptor binding proteins and thus biologically inactive. We measured the actual biologically available IGF-I and found that people with higher levels of IGF-I receptor stimulating activity had a higher prevalence of dementia and to a lesser extent a higher risk of dementia (Chapter 3.5). Hence, we hypothesize that IGF-I increases in response to neuropathology rather than it being involved in the etiology of dementia.

Behavioral and emotional factors

Physical activity is a promising modifiable factor in the etiology of dementia as it stimulates cerebral blood flow and increases neurogenesis.[45,46] Nevertheless, low levels of physical activity might not only be a risk factor but also an early symptom of dementia. To overcome the possibility of reverse causality, we studied the relation between physical activity and dementia over a long follow-up period of up to 13.8 years (Chapter 4.1). We found that higher levels of physical activity were related to a lower risk of dementia only during short follow-up and not during long follow-up. This suggests that reverse causality may indeed play a role, although it is important to keep in mind that our study population consisted of relatively old participants (age range 61-97). Higher levels of physical activity may protect against dementia in younger populations. As discussed before, neuropathologic changes leading to dementia develop many years before the onset of clinical disease, and prevention of dementia through treatment of modifiable risk factors probably needs to be implemented in an early phase of the neuropathological process. Two other factors regarded as risk factors as well as symptoms of dementia are anxiety and depression.[47-49] Investigating both anxiety symptoms and anxiety disorders, we could not establish any associations between anxiety and dementia (Chapter 4.2). Moreover, when we stratified follow-up time into short and long follow-up, results remained similar. We did find an association between anxiety disorders and lower cognitive performance in non-demented participants, but this attenuated after further adjustments. Our results therefore do not support the notion that anxiety is a risk factor of dementia or that anxiety is a very early symptom of dementia. Although anxiety and depression often occur together, we found that the relation between depression and dementia was different from that between anxiety and dementia. We found that depressive symptoms were related to an increased risk of dementia during a follow-up period of 13.7 years (Chapter 4.3). However, we found that depressive symptoms were related to an increased risk of dementia during follow-up to ten years only, and not thereafter. Similar to physical activity, these results therefore suggest that depressive symptoms in elderly people are rather a sign of prediagnostic, and yet to be diagnosed dementia, than a risk factor of dementia.

Methodological considerations

In the following section, I will discuss several methodological issues regarding the work described in this thesis. Since specific issues of separate studies have been mentioned in the corresponding chapters, here I will discuss more general issues of population-based cohort studies in relation to dementia research.

Study design

The research described in this thesis is conducted within the framework of the Rotterdam Study, a large, population-based cohort study that started in 1990 to investigated chronic diseases in the elderly.[50] Conducting dementia research in such a setting offers important advantages. First, population-based studies are less prone to selection bias than clinic-based studies. Second, follow-up for dementia in the original cohort of the Rotterdam Study is complete for over 20 years. Such long follow-up periods are necessary to unravel whether a putative risk factor is indeed a risk factor or merely an early symptom of disease. For example, in chapters 4.1 and 4.3, we have shown that the associations between physical activity and dementia or depression and dementia might be explained via reverse causality, as associations were confined to short follow-up periods. Conversely, a hypothetical drawback of long follow-up periods is that effects could be diluted for risk factors that only have a short-term effect on the etiology of dementia, though not many such risk factors are known.
As in every study design, there are also disadvantages of the Rotterdam Study. Large, prospective, cohort studies are very expensive, which limits data collection. For instance, IGF-I receptor stimulating activity could only be assessed in a subsample of the population, which restricted the power of these analyses. Additionally, some data on putative risk factors could not be collected or was collected only once in a subsequent examination round and not at baseline. The latter might have introduced survival bias in some of our analyses. Also, single measurements are more prone to misclassification as it is likely that they change over time. Furthermore, invasive procedures to collect data, such as lumbar punctures, cannot be easily performed in population-based studies. Finally, the Rotterdam Study population is a fairly homogeneous population, mostly consisting of Caucasians that live in a middle-income district, which limits the generalizability of our results.

Diagnosis of dementia

Participants of the Rotterdam Study were screened for dementia according to a three-step protocol.[19] Additionally, the cohort is constantly monitored for dementia via computerized linkage between the study database and digitized medical records of general practitioners and the Regional Institute for Outpatient Mental Health Care. This enables us to keep following

participants for dementia even when they stop visiting the research center, which restricts selective loss of follow-up of demented participants. Although we could not prevent some loss to follow-up, follow-up for dementia was nearly complete (97.8% for the original cohort and 96.7% for the extended cohort).
DSM-III-R criteria were used to diagnose dementia.[51] Subsequently, we used the NINCDS-ARDRA criteria to sub-classify Alzheimer disease and the NINCDS-AIREN criteria to sub-classify vascular dementia.[52,53] In some clinic-based studies, it is possible to improve the certainty of an Alzheimer disease diagnosis by means of biomarkers of amyloid β deposition and tau phosphorylation.[54] These biomarkers cannot be easily obtained in population-based studies, as they require invasive procedures. However, we know from autopsy studies that besides amyloid β and tau pathology, vascular pathology is also very common in Alzheimer disease.[6] Even more importantly, the majority of patients with dementia have a mixed pathology consisting of Alzheimer pathology, vascular pathology, and Lewy bodies.[55,56] Accordingly, we found that many results of our studies were similar for overall dementia and Alzheimer disease.

Putative risk factors of dementia

Dementia pathology gradually develops over years. Although participants of the Rotterdam Study were eligible from midlife (i.e. 55 years and over), most participants were elderly as the mean age in the original cohort at baseline was 70 years and of the extended cohort 65 years. We know that some risk factors are only related to an increased risk of dementia when assessed at midlife.[16-18] I was not able to test these associations. Moreover, although our follow-up period was relatively long, the pathological process leading to dementia might have started before we assessed the putative risk factors. Therefore I cannot rule out that some associations are explained by reverse causality. Another important consideration is residual confounding. Although I adjusted for several potential confounders, it remains possible that we missed relevant factors. For example, I usually adjusted for the baseline values of confounders, whereas it is very likely that these values changed over time and therefore do not express life-long exposure. Lastly, I mostly investigated modifiable risk factors. However, dementia pathology cannot be fully explained by these factors, as it is likely the result of an interaction between genetic predisposition and environmental factors. Up until now, several genetic variants have been related to an increased risk of late Alzheimer disease, of which *apolipoprotein E (APOE)*-ε4 is the most important. Although genes cannot be modified, it would have been very interesting to study whether the PAR of modifiable dementia risk factors differed between *APOE*-ε4 carriers and non-carriers. Unfortunately, small sample size hampered us to study this. Another study found that the effect of cardiovascular disease was higher in people without an *APOE*-ε4 allele, but this study too was limited by a relatively small

sample size.[57] Hence, studies with larger sample sized are necessary to investigate the effect of the interaction between genes and environmental factors on the risk of dementia.

Clinical implications and suggestions for future research

Our findings that cardiovascular factors are important in dementia have important clinical implications. We provide up to date estimates that a substantial part of dementia patients can be prevented by optimal treatment of cardiovascular risk and improvement of educational level. Also, we found that several markers of subclinical cardiovascular disease were related to an increased risk of dementia, which further underlines the importance of cardiovascular factors. Up until now, dementia subtypes are usually regarded as distinct subtypes with different underlying etiologies. However, from autopsy studies we know that most dementia patients suffer from a mixed pathology.[55,56] Accordingly, in this thesis I showed that cardiovascular factors are not only important in vascular dementia, but also in Alzheimer disease. Clinicians should therefore realize that treatment and prevention of cardiovascular diseases and risk factors is also important for prevention of Alzheimer disease and not only for prevention of vascular dementia. Unfortunately, we were not able to assess the effect of several promising modifiable risk factors, such as low physical activity, depression, unhealthy dietary habits, and less social engagement. Although we showed that low physical activity and depressive symptoms at an elderly age are probably early manifestations instead of risk factors of dementia, they might be risk factors when assessed at midlife. Future studies should therefore investigate whether improvement of other modifiable risk factors adds to the preventive potential of dementia. Moreover, one should keep in mind that our results remain estimates based on observational studies. Clinical trials are currently underway to assess the true effect of optimal treatment of risk factors. Previous trials targeting a single risk factor thus far have only shown a modest effect or were not successful at all.[58,59] However, as dementia is a multifactorial disease, risk factors probably need to be targeted simultaneously before an effect on the risk of dementia can be achieved. Therefore, more recent trials focus on the combination of improving physical activity and strict vascular risk factor control in elderly people at risk for dementia.[60] Another important remark is that dementia is only diagnosed after a long preclinical phase during which neuropathology gradually accumulates. At present, much research focuses on disentangling the earliest signs of disease. Of particular interest in this regard is MCI. We found that several vascular risk factors and MRI-correlates were related to MCI. However, these were cross-sectional analyses and longitudinal studies are required to confirm our results. Furthermore, people with MCI already have symptoms, probably caused by irreversible neuronal damage. Thus, it would be better to identify earlier markers of disease, that is, before people experience complaints. Amyloid β, tau, and cerebrovascular

pathology can be assessed years before clinical symptoms occur.[61,62] However, autopsy studies have shown that these features may also be present in people without dementia.[55,56] More specific markers for dementia are therefore much awaited for. Not only will these markers improve prevention and treatment, they will also aid to unravel the etiology of dementia.

In summary, with this thesis, I have shown that cardiovascular diseases and risk factors are important in dementia, including Alzheimer disease. Targeting cardiovascular risk together with improved education are at present the most viable options for prevention. However, cardiovascular pathology is not the only important feature in dementia nor are amyloid plaques, neurofibrillary tangles, or Lewy bodies. For example, recent studies have proposed a significant role for neuroinflammation.[63,64] Given the complex nature of dementia, it is most likely that different pathways interact in the etiology of the disease. Hence, future studies should focus on a combination of pathways rather than aiming on a single pathway. Probably only then will we be able to fully understand the underlying causes of dementia, which is essential to overcome the growing burden of this devastating disease.

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Chapter 6

Summary

Dementia is the most common neurodegenerative disorder in the elderly and causes a large patient and societal burden. Unfortunately, the etiology of dementia is largely unknown and there is no effective therapy. Currently, dementia is recognized as a major health problem and much research is being undertaken to unravel the etiology, find effective treatments, and identify preventive options for this devastating disease. Alzheimer disease and vascular dementia are the most common subtypes of dementia. Although cardiovascular diseases and risk factors are known to play an important role in vascular dementia, their role in Alzheimer disease is also more and more being acknowledged. Investigating the role of cardiovascular risk in dementia is important as it might provide an opportunity for prevention. However, preventive options probably need to be targeted timely as dementia is only diagnosed after a long preclinical phase. This necessitates identification and investigation of early phases of the disease, such as mild cognitive impairment (MCI).

The aim of this thesis was to assess the impact of known risk factors of dementia and to identify emerging risk factors. All research was conducted within the Rotterdam Study, a large prospective, population-based study among people 55 years of age and older residing in Ommoord, a district of Rotterdam, the Netherlands.

Chapter 2 focuses on the impact of established risk factors. In **Chapter 2.1**, I concluded there is abundant evidence for a role of cardiovascular diseases and risk factors in the etiology of Alzheimer disease. Not only clinical factors, but also markers of subclinical vascular disease, such as large vessel disease and cerebral small vessel disease are related to an increased risk of Alzheimer disease. These findings suggest that dementia could potentially be prevented by optimal treatment of cardiovascular diseases and risk factors. Indeed, in **Chapter 2.2**, I estimated that about a quarter to a third of dementia cases could be prevented by optimal treatment of cardiovascular risk and improvement of educational level. Furthermore, we found that this potential for prevention had not declined over the last decades. Therefore, these findings call for urgent public health interventions. In **Chapter 2.3**, I investigated determinants, MRI-correlates and the prognosis of MCI, that defines the phase between normal aging and dementia. I found that about 10% of our population-based sample had MCI (**Chapter 2.3**). Additionally, I assessed that cardiovascular risk factors and MRI-correlates of cerebral vascular disease were related to MCI, which in turn was associated with an increased risk of dementia and mortality. These findings therefore underline the importance of vascular factors even in the early phases of dementia.

In **Chapter 3**, I focused on emerging risk factors of dementia. It has been disputed whether atrial fibrillation is a risk factor of dementia, since both diseases may also be related through shared etiology. Within our study population, atrial fibrillation was related to an increased risk of dementia over a follow-up period of 20 years (**Chapter 3.1**). Furthermore, I found that the longer people suffered from atrial fibrillation, the higher their risk on dementia was. However, these associations were confined to the younger part of our population, suggesting

that atrial fibrillation needs to start at a younger age to contribute to the etiology of dementia. Besides clinical cardiovascular disease, I also investigated subclinical measures of cardiovascular disease. Using echocardiography measures, I found that subclinical cardiac dysfunction was related to an increased risk of stroke and dementia (**Chapter 3.2**). Interestingly, diastolic function was related to stroke and dementia whereas systolic function was related to an increased risk of stroke only. In addition, worse diastolic function was related to silent infarcts, especially lacunar infarcts. Hence, it should be further investigated whether diastolic function, rather than systolic function, is more important in the etiology of dementia. Another marker of subclinical cardiovascular disease is atherosclerosis. Larger calcification volumes in the aortic arch, and extracranial and intracranial arteries were related to an increased risk of dementia (**Chapter 3.3**). Calcification volumes in all vessel beds were associated with an increased risk of cognitive decline in non-demented people, suggesting that generalized atherosclerosis, rather than localized atherosclerosis, is related to dementia. Therefore, reducing atherosclerosis might be another potential target for prevention. A measure closely related to atherosclerosis is cerebral vasomotor reactivity, which reflects the ability of the cerebral arterioles to dilate in the event of hypercapnia to improve cerebral blood flow. Lower cerebral vasomotor reactivity was related to an increased risk of mortality, especially cardiovascular mortality (**Chapter 3.4**). However, future studies should investigate whether lower cerebral vasomotor reactivity is also related to an increased risk of dementia. Besides cardiovascular factors, in **Chapter 3.5**, I also investigated a metabolic factor in relation to dementia. Insulin-like growth factor-I (IGF-I) has several neuroprotective features and it has been suggested that lower IGF-I is related to an increased risk of dementia. However, we measured the actual biologically available IGF-I and found that people with higher levels of IGF-I receptor stimulating activity had a higher prevalence of dementia and to a lesser extent, a higher risk of dementia. This suggests that IGF-I increases in response to neuropathology rather than it being involved in the etiology of dementia.

The associations of physical activity, anxiety, and depression with dementia are described in **Chapter 4**. These behavioral and emotional factors might related to an increased risk of dementia, but they are also symptoms of the disease. To overcome the possibility of reverse causality, long follow-up studies are required. In our study, both lower levels of physical activity and presence of depressive symptoms were related to an increased risk of dementia only during shorter follow-up and not during longer follow-up (**Chapters 4.1 and 4.3**). This suggests reverse causality, however, it is important to mention that we only investigated elderly people. Thus, it is possible that lower levels of physical activity and presence of depressive symptoms are risk factors of dementia when assessed in midlife. Although depression and anxiety often occur together in demented people, anxiety was not related to an increased risk of dementia (**Chapter 4.2**). This association did not differ over time, suggesting that anxiety is not a risk nor a very early symptom of dementia.

In **Chapter 5**, I discuss the main findings and methodological considerations regarding the studies. In addition, I discuss the clinical implications of the work and give recommendations for future research.

Dementie is de meest voorkomende neurodegeneratieve aandoening bij ouderen en veroorzaakt een hoge belasting van zowel de patiënt als de maatschappij. De oorzaak van dementie is nog grotendeels onbekend en de ziekte is niet te genezen. Door de vergrijzing van de populatie is de verwachting dat het aantal dementie patiënten in de komende jaren fors zal stijgen. Momenteel wordt er veel onderzoek gedaan om de oorzaak van dementie te ontrafelen, therapeutische mogelijkheden te ontwikkelen en mogelijkheden voor preventie te ontdekken. De ziekte van Alzheimer en vasculaire dementie zijn de twee meest voorkomende subtypes van dementie. Cardiovasculaire factoren zijn van belang bij het ontstaan van vasculaire dementie, maar tegenwoordig weten we dat deze factoren ook een rol spelen in de etiologie van de ziekte van Alzheimer. Het is belangrijk om het effect van cardiovasculaire factoren op het ontstaan van dementie verder te onderzoeken omdat dit mogelijkheden zou kunnen bieden voor preventie. Op het moment dat de ziekte wordt gediagnosticeerd is er al veel irreversibele schade in de hersenen, die zich langzaam over jaren heeft ontwikkeld. Preventieve opties moeten daarom waarschijnlijk in een vroeg stadium worden gestart wat het van belang maakt om vroege stadia van dementie te identificeren en onderzoeken.

Het doel van dit proefschrift was om het effect van bekende risicofactoren op dementie in kaart te brengen en om nieuwe risicofactoren te ontdekken. Al het onderzoek in dit proefschrift maakt deel uit van het Erasmus Rotterdam Gezondheid Onderzoek (ERGO). Dit is een grote populatie studie onder mensen van 55 jaar en ouder die in de Rotterdamse wijk Ommoord wonen en vanaf 1990 gevolgd worden.

In **hoofdstuk 2** heb ik me gericht op het effect van bekende risicofactoren. In **hoofdstuk 2.1** heb ik de literatuur onderzocht en ben ik tot de conclusie gekomen dat er veel bewijs is voor een rol van cardiovasculaire ziekten en risicofactoren in de etiologie van de ziekte van Alzheimer. Niet alleen klinische ziekten, maar ook markers van subklinische cardiovasculaire ziekte zijn gerelateerd aan het ontstaan van de ziekte van Alzheimer. Dit suggereert dat dementie eventueel voorkómen zou kunnen worden door optimale behandeling van cardiovasculaire ziekten en risicofactoren. Inderdaad, in **hoofdstuk 2.2** vond ik dat een kwart tot een derde van het aantal dementie patiënten kan worden voorkómen door optimale behandeling van deze factoren en verbetering van het opleidingsniveau. Ook vond ik dit percentage gelijk is gebleven in de afgelopen twintig jaar. Deze bevindingen nodigen daarom uit tot intensievere maatregelen van de gezondheidszorg om het cardiovasculaire risicoprofiel van de populatie te verbeteren. Een belangrijk punt is dat deze preventieve opties tijdig moeten worden geïmplementeerd. In dit kader heb ik in **hoofdstuk 2.3**, mild cognitive impairment (MCI) onderzocht, een klinisch syndroom dat een voorstadium kan zijn van dementie. Ongeveer 10% van onze studie populatie had MCI en wij vonden dat verschillende cardiovasculaire risicofactoren en maten van vasculaire ziekte op beeldvorming waren gerelateerd aan MCI. Mensen met MCI hadden een verhoogd risico op dementie en overlijden. Deze bevindingen onderstrepen daarom het belang van vasculaire factoren, zelfs in de vroege stadia van dementie.

In **hoofdstuk 3** staan verschillende opkomende risicofactoren van dementie beschreven. Ondanks dat cardiovasculaire ziekten een rol spelen in de etiologie van dementie, is er nog veel onduidelijkheid over de rol van atriumfibrilleren. Atriumfibrilleren zou een risicofactor van dementie kunnen zijn, maar aan de andere kant is het ook mogelijk dat beide ziekten naast elkaar bestaan omdat ze veel dezelfde risicofactoren hebben. Ik vond dat atriumfibrilleren was gerelateerd aan een verhoogd risico op dementie over een tijdsperiode van 20 jaar (**hoofdstuk 3.1**). Verder vond ik dat hoe langer het atriumfibrilleren bestond, hoe hoger het risico op dementie was. Deze resultaten waren echter alleen zichtbaar in de jongere deelnemers (jonger dan 67 jaar), wat zou kunnen betekenen dat atriumfibrilleren op jongere leeftijd moet ontstaan om bij te dragen aan het ontstaan van dementie. Naast klinische cardiovasculaire ziekten, heb ik ook markers van subklinische cardiovasculaire ziekte onderzocht. Mensen die geen klinische cardiovasculaire ziekte hadden, maar wel een slechtere functie van hun hart, hadden een verhoogd risico op een beroerte en op dementie (**hoofdstuk 3.2**). Hierbij was het opvallend dat een verminderde diastolische functie was gerelateerd aan zowel een verhoogd risico op een beroerte als op dementie, terwijl een verminderde systolische functie alleen was gerelateerd aan een verhoogd risico op een beroerte. Verder was een verminderde diastolische functie gerelateerd aan stille herseninfarcten, met name lacunaire infarcten. Toekomstige studies zullen moeten uitwijzen of diastolische hartfunctie belangrijker is voor het ontstaan van dementie dan systolische hartfunctie. Een andere marker van subklinische cardiovasculaire ziekte is atherosclerose. Verkalking van de aortaboog en van de extracraniële en intracraniële arteriën was gerelateerd aan een verhoogd risico op dementie (**hoofdstuk 3.3**). Verkalking in alle vaatbedden was geassocieerd met een verhoogd risico op cognitieve achteruitgang in mensen zonder dementie. Dit impliceert dat gegeneraliseerde atherosclerose bijdraagt aan een verhoogd risico op dementie. Vermindering van atherosclerose zou daarmee de preventie van dementie kunnen beïnvloeden. Een marker die sterk gerelateerd is aan atherosclerose is de reactiviteit van de cerebrale vaten. In **hoofdstuk 3.4** vonden we dat een verminderde reactiviteit geassocieerd was met een verhoogd risico op overlijden. Of een verminderde reactiviteit van de cerebrale vaten ook gerelateerd is aan een verhoogd risico op dementie moet echter nog worden onderzocht. In **hoofdstuk 3.5** heb ik de relatie tussen insulin-like growth factor (IGF)-I en dementie bestudeerd. Ik vond dat mensen met een hogere fractie van de actieve vorm van IGF-I vaker dement waren. Ook vond ik dat een hogere fractie van het actieve IGF-I gerelateerd was aan een verhoogd risico op dementie. Echter, dit verband was minder sterk, wat het waarschijnlijker maakt dat IGF-I stijgt als gevolg van dementie in plaats van dat het betrokken is bij het ontstaan van de ziekte.

Hoofdstuk 4 richt zich op de verbanden tussen fysieke activiteit, angst en depressie met dementie. Deze factoren zouden aan de ene kant risicofactoren van dementie kunnen zijn, maar zijn aan de andere kant ook symptomen van de ziekte. De verbanden tussen deze factoren en dementie moeten daarom worden onderzocht over een lange tijdsperiode. In onze studie

vonden we dat zowel verminderde fysieke activiteit als depressieve klachten alleen op de korte termijn waren geassocieerd met een verhoogd risico op dementie, en niet op de lange termijn (**hoofdstukken 4.1 en 4.3**). Dit suggereert dat verminderde fysieke activiteit en depressieve klachten eerder vroege symptomen zijn van dementie, dan dat ze risicofactoren zijn. Echter, onze studiepopulatie bestond uit ouderen en het is heel goed mogelijk dat verminderde fysieke activiteit en depressieve klachten op middelbare leeftijd wel risicofactoren zijn van dementie. Ondanks het feit dat depressie en angst vaak samen voorkomen bij demente patienten, vond ik dat angst niet gerelateerd was aan een verhoogd risico op dementie, ook niet op de korte termijn (**hoofdstuk 4.2**). Dit zou kunnen betekenen dat angst geen risicofactor of vroeg symptoom is van de ziekte.

In **hoofdstuk 5** heb ik de belangrijkste bevindingen en methodologische overwegingen van de studies bediscussieerd. Daarnaast bepreek ik in dit hoofdstuk de klinische implicaties van de studies en geef ik suggesties voor toekomstig onderzoek.

Chapter 7

Dankwoord

List of publications

PhD portfolio

About the author

Dankwoord

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PhD portfolio

Name: Renée Frederica Anna Gertrude de Bruijn
Erasmus Medical Center Departments Epidemiology and Neurology
PhD period: 2011-2014
Research schools: NIHES, COEUR
Promotors: Prof.dr. A. Hofman, Prof.dr. P.J. Koudstaal

Research skills

2011-2013: MSc in Clinical Epidemiology, Netherlands Institute for Health Sciences, Erasmus University Rotterdam, the Netherlands (including courses on methodology, study design, and statistical analysis)

General academic skills

2014: Biomedical English Writing and Communication, Erasmus Medical Center, Rotterdam, the Netherlands

In-depth courses

2011: Course on SNPs and Human Diseases, Molecular Medicine, Erasmus Medical Center, Rotterdam, the Netherlands
2012: Physical Activity and Nutrition, Cambridge University, Cambridge, United Kingdom

International conferences

2011: Alzheimer's Association International Conference, Paris, France
2012: Alzheimer's Association International Conference, Vancouver, Canada. Oral presentation.
2014: American Academy of Neurology Annual Meeting, Philadelphia, United States of America. Two poster presentations.
2014: Alzheimer's Association International Conference, Copenhagen, Denmark. Poster presentation.

Reviewing activities

2013-2014: Reviewing papers for international scientific journals

Teaching activities

2014: Supervising practicals at NIHES, Erasmus Medical Center, Rotterdam, the Netherlands

About the author

Renée Frederica Anna Gertrude de Bruijn was born on January 29, 1985, Breda, the Netherlands. After graduating at the Sint Oelbert Gymnasium, Oosterhout in 2003, she attended the James Boswell Institute, Utrecht, to obtain certificates in physics, chemistry, and mathematics. Subsequently, in 2004, she started her medical education at the Erasmus University in Rotterdam. After graduating in 2010, she worked as a resident at the department of Neurology at the Erasmus Medical Center (prof.dr. P.A.E. Sillevis Smitt). In July 2011, she began working at the department of Epidemiology on the project described in this thesis under the supervision of prof.dr. A. Hofman (department of Epidemiology) and prof.dr. P.J. Koudstaal (department of Neurology). The abstract "Cerebral vasomotor reactivity and the risk of mortality: the Rotterdam Study", she wrote together with Marileen Portegies, was awarded the Young Investigator Award at the 2013 European Stroke Conference. In August 2013, she obtained a Master of Health Sciences in Clinical Epidemiology at the Netherlands Institute for Health Sciences. As of January 2015, she is working as a resident at the department of Neurology at the Erasmus Medical Center (prof.dr. P.A.E. Sillevis Smitt).