

Inflammation, Vascular Factors
and Risk of Dementia

Marieke van Oijen



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Inflammation, Vascular Factors and Risk of Dementia

Ontsteking, vasculaire factoren en kans op dementie

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Voor mijn ouders





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Manuscripts based on the studies described in this thesis

Chapter 2.1

Van Oijen M, van der Meer IM, Witteman JCM, Hofman A, Koudstaal PJ, Breteler MMB. Lipoprotein-associated phospholipase A2 is associated with risk of dementia. *Annals of Neurology*. 2006 Jan;59 (1):139-144

Chapter 2.2

Van Oijen M, Witteman JCM, Hofman A, Koudstaal PJ, Breteler MMB. Fibrinogen is associated with increased risk of Alzheimer's disease and vascular dementia. *Stroke*. 2005 Dec;36 (12):2637-41

Chapter 2.3

Van Oijen M, de Maat MP, Kardys I, de Jong FJ, Hofman A, Koudstaal PJ, Witteman JCM, Breteler MMB. Polymorphisms and haplotypes in the C-reactive protein gene and dementia. *Neurobiology of Aging*. (in press)

Chapter 2.4

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Chapter 2.5

Van Oijen M, Cheung EYL, de Jong FJ, Hofman A, Koudstaal PJ, de Maat MP, Breteler MMB. Haplotypes in the fibrinogen gene and dementia.

Chapter 2.6

Van Oijen M, Arp PP, de Jong FJ, Hofman A, Koudstaal PJ, Uitterlinden AG, Breteler MMB. Polymorphisms in the interleukin 6 and transforming growth factor $\beta 1$ gene and risk of dementia. The Rotterdam Study. *Neuroscience Letters*. 2006 Jul 10; 402(1-2): 113-7

Chapter 3.1

Van Oijen M, de Jong FJ, Hofman A, Koudstaal PJ, Witteman JCM, Breteler MMB. Atherosclerosis and risk of dementia. *Submitted*

Chapter 3.2

Ruitenbergh A, van Oijen M, van Swieten JC, Koudstaal PJ, Witteman JCM, Hofman A, Breteler MMB. Blood pressure and the risk of dementia. Results from the Rotterdam Study.





Chapter 3.3

Poels MMF, van Oijen M, Mattace-Raso FUS, Hofman A, Koudstaal PJ, Witteman JCM, Breteler MMB. Arterial stiffness, cognitive decline and risk of dementia. The Rotterdam Study. *Stroke*. (*in press*).

Chapter 3.4

Van Oijen M, Ikram MA, de Jong FJ, Kors JA, Hofman A, Koudstaal PJ, Witteman JCM, Breteler MMB. Unrecognized myocardial infarction and the risk of dementia. *Submitted*

Chapter 4.1

Van Oijen M, de Jong FJ, Hofman A, Koudstaal PJ, Breteler MMB. Subjective memory complaints, education and dementia. *Submitted*

Chapter 4.2

Van Oijen M, Soares HD, Hofman A, Koudstaal PJ, Breteler MMB. Plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ and the risk of dementia. *Lancet Neurology*. 2006 Aug; 5(8): 655-60





Chapter 1. Introduction

Chapter 1. Introduction

Dementia is a common and devastating condition in the elderly. The prevalence ranges from approximately 1% in 60-year-old persons to more than 30% in persons over 85 years¹ and the burden of disease is likely to rise as life expectancy increases. The clinical syndrome of dementia is characterized by a decline in memory and other cognitive functions, interfering with activities of daily living. Different subtypes of dementia are recognized, Alzheimer's disease being the most common subtype followed by vascular dementia. It is likely that sporadic late onset dementia is a result of interplay of both genetic and environmental factors but the precise etiology is not yet known. Several mechanisms may cause brain pathology that ultimately causes the syndrome of dementia. Of particular interest are potentially modifiable mechanisms, including inflammatory and vascular mechanisms. Inflammation in the brain could contribute to or promote neuronal loss² and also peripheral inflammation may contribute to the pathogenesis of dementia.^{3,4} Also, there is evidence for a prominent role of vascular disease and vascular factors in the development of dementia, both vascular dementia and Alzheimer's disease.^{5,6} The association between vascular disease and dementia may be explained by overt cerebrovascular disease, such as stroke or cerebral small vessel disease,⁷ or result from cerebral hypoperfusion.⁵ Furthermore, inflammation has been linked to vascular disease and vascular risk factors and it is conceivable that both inflammatory and vascular mechanisms represent a similar pathway or interact in their association with dementia. Though associations of inflammatory and vascular factors with dementia have been reported, most existing evidence comes from cross-sectional studies or studies with a short follow-up period. Because it is likely that the neuropathology underlying the dementia process occurs many years before the clinical syndrome is recognized, it cannot be concluded from these studies that inflammatory and vascular factors actually cause dementia. Therefore, prospective studies with a long follow-up period are needed to establish whether inflammatory and vascular factors are actually causes and not consequences of the dementia process or simply unrelated co-existent disorders. Furthermore, a longterm follow-up study allows identification of markers in the extended period preceding the clinical syndrome of dementia. These preclinical markers may be useful for early detection of dementia. The main objective of the research described in this thesis was to examine the role of inflammatory and vascular factors in the pathogenesis of dementia using a long follow-up. A secondary objective was to identify markers that may be of help in early detection of dementia. All research was performed in the Rotterdam Study, a large prospective, population-based cohort study among 7,983 persons of 55 years and over who have been followed since 1990.





Information on inflammatory markers, vascular disease and risk factors was obtained at baseline and updated during follow-up examinations. From the start of the study new dementia patients were identified by means of a multi-step cognitive screening protocol conducted at the research center and linkage of the study database with medical records of general practitioners and the outpatient mental health care. First, I examined the influence of inflammation. I investigated the association of plasma levels of inflammatory markers, lipoprotein-associated phospholipase A2 (chapter 2.1), fibrinogen and C-reactive protein (chapter 2.2) with the risk of dementia. Levels of inflammatory markers are partly genetically determined, and studying variation in inflammatory genes may provide evidence for a causal role of inflammation in the pathogenesis of dementia. Therefore, common variation in genes encoding C-reactive protein was examined in relation to risk of dementia in chapter 2.3. In chapter 2.4, I investigated the association between fibrinogen plasma levels and cerebral small vessel disease on brain imaging. Also, I examined the relation of common variation in fibrinogen genes believed to be involved in regulating the fibrin clot structure, with cerebral small vessel disease (chapter 2.4) and dementia (chapter 2.5). I studied the influence of variation in genes that influence levels of two inflammatory cytokines that may contribute to the formation of Alzheimer pathology, interleukin 6 and transforming growth factor β 1, in chapter 2.6. Next, I examined the influence of several vascular factors on risk of dementia; atherosclerosis (chapter 3.1), blood pressure (chapter 3.2), arterial stiffness (chapter 3.3) and myocardial infarction (chapter 3.4).

In chapter 4, I investigated potential markers for early detection of dementia. Self-perceived cognitive deficits, expressed as subjective memory complaints, may be the first sign of cognitive decline in early dementia, especially in highly educated persons. I examined whether the association between subjective memory complaints and risk of dementia was influenced by level of education in chapter 4.1. Since levels of amyloid β in the brain are thought to play an important role in dementia, I examined whether plasma amyloid β levels could serve as a marker of incipient dementia (chapter 4.2). In the final chapter (chapter 5) I reviewed my main findings and their implications.





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Chapter 2. Inflammation and dementia

Chapter 2.1

Lipoprotein-associated phospholipase A2 is associated with risk of dementia

Abstract

High levels of the inflammatory marker lipoprotein-associated phospholipase A2 (Lp-PLA2) have been proposed a predictor of coronary heart disease and stroke. Since both inflammation and vascular disease are associated with dementia, we examined the association between Lp-PLA2 and the risk of dementia. Within the Rotterdam Study, a population-based prospective cohort study, we performed a case-cohort study. Of the 6,713 participants at risk for dementia, a random sample of 1,742 individuals was drawn. During follow-up (mean 5.7 years), 302 incident dementia cases were identified. Cox' proportional hazard models were used to estimate the association of Lp-PLA2 and dementia. We found that persons with higher levels of Lp-PLA2 had an increased risk of dementia. Compared to the first quartile of Lp-PLA2, age and sex adjusted hazard ratios (95% confidence interval) for dementia for the second, third and fourth quartiles were 1.19 (0.78-1.81), 1.15 (0.74-1.79) and 1.56 (1.03-2.37), respectively (p-value for trend 0.04). Additional adjustment for cardiovascular and inflammatory factors did not change the estimates. This is the first study that shows that Lp-PLA2 is associated with the risk of dementia independent of cardiovascular and inflammatory factors and provides evidence for a potential role of Lp-PLA2 in identifying persons at risk for dementia.

Introduction

Lipoprotein-associated phospholipase A2 (Lp-PLA2, also known as platelet-activating factor acetylhydrolase) has been proposed as a predictor of coronary heart disease independent of traditional cardiovascular risk factors.¹⁻⁴ Lp-PLA2 circulates in the blood, associated with LDL-cholesterol.⁵ It hydrolyzes oxidized phospholipids to generate lysophosphatidylcholine and oxidized fatty acids, which are believed to have proinflammatory properties.⁶ Lp-PLA2 may be an inflammatory marker or directly promote atherogenesis.⁷ Recently our group reported on the association of higher levels of Lp-PLA2 not only with coronary heart disease but also with stroke.⁸ Since inflammation markers,^{9,10} cardiovascular risk factors^{11,12} and cerebrovascular disease^{13,14} all may play a role in dementia, we hypothesized a relation between Lp-PLA2 levels and the risk of dementia. We investigated the association of Lp-PLA2 and risk of dementia in the Rotterdam Study among people aged 55 years and over.





Methods

Study population

The Rotterdam Study is a population-based prospective cohort study that investigates the incidence and risk factors of cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases in the elderly.¹⁵ From 1990-1993, all 10,275 residents aged 55 years or over of Ommoord, a district of the city of Rotterdam, were invited to participate in an extensive home interview and two visits to the research center, and 7,983 (78%) of them agreed. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. At the baseline clinical examination, blood samples were drawn from 7,050 persons of whom 7,047 underwent screening for dementia. In these, prevalent dementia was diagnosed in 334 resulting in a cohort with blood samples taken and at risk for dementia of 6,713 persons. Follow-up examinations were conducted in 1993-1994 and in 1997-1999. In addition, through linkage with records of general practitioners, the total cohort was continuously monitored for major disease outcome. This resulted in a virtually complete follow-up until January 1, 2000.

Study design

For reasons of efficiency a case-cohort design^{16,17} was used. In this design, a random sample, or “subcohort”, is drawn from the source population. Persons from the source population who are not included in the subcohort yet develop the disease, are selected as additional cases and added to the analyses. Baseline exposure is measured in the cases and controls included in the subcohort and in the additional cases. In the case-cohort analysis only persons from the random cohort contribute to follow-up time. A random subcohort of 1,742 persons was drawn from our cohort at risk, of whom 77 developed dementia during follow-up (figure 1). We added 225 dementia patients who developed dementia outside the subcohort. Of the total of 302 incident dementia patients, 222 were diagnosed with Alzheimer’s disease (AD), including 22 patients with AD accompanied by cerebrovascular disease, 44 with vascular dementia, 14 patients with dementia in Parkinson’s disease and 22 patients with dementia due to other causes such as multisystem atrophy, frontotemporal dementia and Lewy body dementia.

Measurement of Lp-PLA2 activity

Plasma aliquots prepared from non-fasted blood samples were collected at baseline and stored at -80°C, and Lp-PLA2 activity was measured with a high throughput radiometric activity assay as described. Briefly, plasma samples were divided into aliquots, placed in 96-well microtiter plates and mixed with a substrate solution consisting of 0.4 µmol/L [3H]-PAF (specific activity 21.5 Ci/mmol, Perkin Elmer Life Sciences) and 99.6 µmol/L C16-PAF (Avanti Polar Lipids Inc) in assay buffer (100 mmol/L HEPES, 150 mmol/L NaCl, 5mmol/L



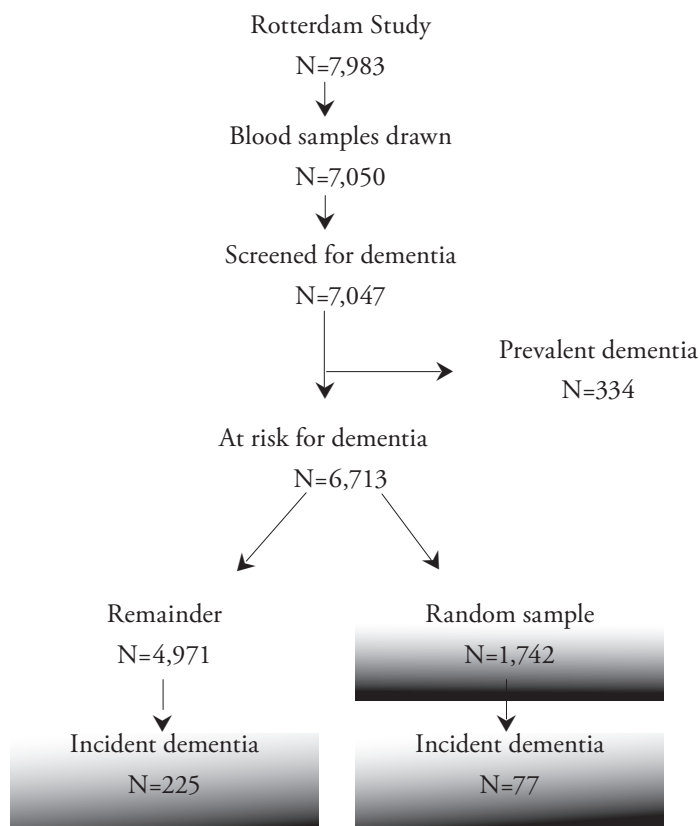


Figure 1. Description of study population. In the case-cohort analysis, the random sample, incident cases arising from the random sample and additional incident cases are included (shaded areas).

EDTA, pH 7.4). The reactions were allowed to proceed at room temperature for 5 min before sequestering of the phospholipid substrates by an ice-cold fatty acid-free bovine serum albumin solution at a final concentration of 16.1 mg/mL. The BSA-lipid complexes were then precipitated with ice-cold trichloroacetic acid at a final concentration of 7.8% and pelleted by centrifugation at $\sim 6,000$ g for 15 min at 4°C. Aliquots of the supernatant containing the reaction products were transferred to another microplate (Perkin Elmer) and the radioactivity counted in a Topcount liquid scintillation counter (Perkin Elmer Life Sciences) upon addition of Microscint-20 scintillation cocktail (Perkin Elmer Life Sciences). Lp-PLA2 activity was expressed as nanomoles of PAF hydrolyzed per minute per 1 mL of plasma samples. On the basis of split samples, the coefficient of variation was 8.4%. Specimens of cases were assessed in the same runs as the random sample from the subcohort.



Covariates

At baseline a trained investigator visited all participants at home and collected information using a computerized questionnaire. The interview included current health status, medical history, drug use, and smoking behavior. Additionally, during two visits to the research center, clinical measures were obtained. Height and weight were measured and the body mass index (BMI) was calculated (weight (kg)/length (m²)). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. Non-fasting blood samples were drawn and immediately frozen. Total cholesterol, high density lipid (HDL)-cholesterol and glucose were measured within 2 weeks, as described previously.¹⁸ Immediately after blood sampling, white cell count was assessed in citrate plasma using a Coulter Counter T540[®] (Coulter electronics, Luton, England), which has a coefficient of variation less than 2.0%. Quality of assessments was continuously monitored by Instruchemi[®] (Hilversum, the Netherlands). Using a nephelometric method (Image[®], Beckman Coulter), high sensitivity C-reactive protein was measured in blood samples that were kept frozen at -20 °C. Genotyping for APOE was performed on coded DNA specimens without knowledge of diagnosis. A polymerase chain reaction was performed.¹⁹ Two groups were formed on the basis of presence or absence of an APOE ε4 allele. Furthermore, ultrasonography of both carotid arteries was performed. We used presence of carotid plaques as an indicator of atherosclerosis. Carotid plaques were determined at six different locations in the carotid arteries: common carotid artery, carotid bifurcation, and internal carotid artery at both left and right side.²⁰ We defined diabetes mellitus as a random or postload glucose level ≥ 11.1 mmol/l or the use of blood glucose lowering medication.

Diagnosis of dementia

During baseline and follow-up examinations the diagnosis of dementia followed a similar three-step protocol.²¹ Two brief tests of cognition (Mini-Mental State Examination (MMSE)²² and Geriatric Mental State schedule (GMS) organic level²³ were used to screen all subjects. Screen-positives (MMSE score < 26 or GMS organic level > 0) underwent further cognitive testing using the Cambridge examination for mental disorders of the elderly (Camdex).²⁴ Persons who were suspected of having dementia were subsequently examined by a neurologist, a neuropsychologist and, if possible, had a MRI scan. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.²¹ The diagnoses of dementia and Alzheimer's disease were made in accordance with internationally accepted criteria for dementia (DSM-III-R),²⁵ Alzheimer's disease (NINCDS-ADRDA),²⁶ and vascular dementia (NINDS-AIREN)²⁷ by a panel of a neurologist, neuropsychologist and research physician who reviewed all existing information.





Data analysis

The association of Lp-PLA2 activity with dementia and subtypes of dementia was evaluated in a case-cohort design through Cox' proportional hazard models with modification of the standard errors based on robust variance estimates. We used the method according to Barlow in which the random cohort is weighted by the inverse of the sampling fraction from the source population.^{16, 17} Lp-PLA2 was normally distributed and first we looked at the association of Lp-PLA2 and the risk of dementia and subtypes of dementia entering Lp-PLA2 as a linear term (per standard deviation (SD)) in the model. Then, quartiles of Lp-PLA2 were made and the lowest quartile was used as the reference category. Analyses were adjusted for age and sex, and additionally for the following confounders; BMI, total cholesterol, HDL-cholesterol, systolic blood pressure, carotid plaques, current smoking, diabetes mellitus, white cell count and C-reactive protein. Adjustment was made for presence of the APOE ε4 allele. In the test for trend analysis we replaced quartiles of Lp-PLA2 activity by continuous values of Lp-PLA2 activity. To investigate whether the association between Lp-PLA2 and dementia was different for men and women, or varied with cholesterol levels, we looked at interactions of Lp-PLA2 and sex, and Lp-PLA2 and cholesterol. In analyses stratified by sex we used sex specific quartiles. To assess whether the association between Lp-PLA2 and dementia was mediated by stroke, we repeated the analyses excluding persons with prevalent stroke and censoring those with incident stroke. To assess whether the association was influenced by lipid-lowering medication, in an additional analysis we excluded persons who used lipid-lowering medication (statins or fibrates) at baseline. We had missing values for covariates in less than 5% (7.5% in C-reactive protein). We imputed the median value for missing values. Data analyses were performed using SAS 8.2 and SPSS 11.0 statistical software.

Results

Characteristics of the random cohort are shown in table 1. Increasing levels of Lp-PLA2 were associated with an increased risk of dementia (table 2). People in the upper quartile had a 56% higher risk compared to those in the lower quartile. Additional adjustments for BMI, total cholesterol, HDL-cholesterol, systolic blood pressure, carotid plaques, current smoking, diabetes mellitus, white cell count and C-reactive protein did not markedly affect the estimates, nor did additional adjustment for presence of APOE ε4 allele. We looked at the associations in men and women separately (table 3). The relation between Lp-PLA2 and risk of dementia seemed more pronounced in men. However, the p-value of the interaction term between sex and Lp-PLA2 activity was 0.07.





Table 1. Characteristics of the random cohort (n=1,742) and the total cohort at risk (n=6,713)

Variable	Total cohort at risk	Random cohort
Age (years) (SD)	69.5 (9.1)	68.5 (8.6)
Women (%)	59.9	61.0
Body mass index (kg/m ²) (SD)	26.3 (4.0)	26.2 (3.6)
Systolic blood pressure (mm Hg) (SD)	139.3 (22.3)	138.1 (22.2)
Diastolic blood pressure (mm Hg) (SD)	73.6 (11.5)	73.4 (11.1)
Total cholesterol (mmol/l) (SD)	6.6 (1.2)	6.7 (1.2)
High density lipid-cholesterol (mmol/l) (SD)	1.3 (0.4)	1.3 (0.4)
Diabetes (%)	10.0	9.5
Smokers (%)		
Current	22.8	23.1
Former	41.6	41.0
Lp-PLA2 activity (nmol/min/ml plasma) (SD)	-	44.5 (11.5)

Table 2. Hazard ratio (95% confidence interval) for dementia

	Model 1	Model 2
Lp-PLA2 per SD increase	1.12 (0.97-1.28)	1.16 (0.97-1.37)
1 st quartile	1.00 (reference)	1.00 (reference)
2 nd quartile	1.19 (0.78-1.81)	1.24 (0.80-1.92)
3 rd quartile	1.15 (0.74-1.79)	1.23 (0.76-1.98)
4 th quartile	1.56 (1.03-2.37)	1.74 (1.07-2.83)
Ptrend	0.04	0.03

Model 1; hazard ratio (95% confidence interval) adjusted for age and sex

Model 2; hazard ratio (95% confidence interval) adjusted for body mass index, total cholesterol, high density lipid-cholesterol, systolic blood pressure, carotid plaques, current smoking, diabetes mellitus, white cell count and C-reactive protein

We found no indication that the relation between Lp-PLA2 and dementia varied with cholesterol levels (p-value interaction term between total cholesterol and Lp-PLA2 activity 0.14). The p-value of the interaction term between HDL-cholesterol and Lp-PLA2 was 0.77. No differences between men and women were found.

When we investigated subtypes of dementia, the association of Lp-PLA2 appeared stronger with vascular dementia than with AD (table 4). No clear sex effect was found for these subtypes of dementia.



Table 3. Hazard ratio (95% confidence interval) for dementia by sex

Lp-PLA2	Women		Men	
	Model 1	Model 2	Model 1	Model 2
per SD increase	1.01 (0.85-1.21)	1.06 (0.84-1.33)	1.29 (1.03-1.63)	1.22 (0.93-1.60)
1 st quartile	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
2 nd quartile	1.17 (0.69-1.99)	1.28 (0.74-2.24)	0.90 (0.43-1.88)	0.90 (0.39-2.06)
3 rd quartile	1.20 (0.70-2.07)	1.42 (0.77-2.61)	1.55 (0.77-3.13)	1.35 (0.64-2.83)
4 th quartile	1.21 (0.71-2.06)	1.50 (0.80-2.82)	1.77 (0.92-3.14)	1.51 (0.70-3.25)
Ptrend	0.53	0.23	0.04	0.19

Model 1; hazard ratio (95% confidence interval) adjusted for age and sex

Model 2; hazard ratio (95% confidence interval) adjusted for body mass index, total cholesterol, high density lipid-cholesterol, systolic blood pressure, carotid plaques, current smoking, diabetes mellitus, white cell count and C-reactive protein

Table 4. Hazard ratio (95% confidence interval) by dementia subtype

Lp-PLA2	Alzheimer's disease		Vascular dementia	
	Model 1	Model 2	Model 1	Model 2
per SD increase	1.06 (0.91-1.24)	1.09 (0.90-1.32)	1.20 (0.92-1.57)	1.18 (0.88-1.82)
1 st quartile	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
2 nd quartile	1.12 (0.71-1.77)	1.16 (0.72-1.84)	0.82 (0.24-2.84)	0.78 (0.22-2.73)
3 rd quartile	1.00 (0.61-1.62)	1.03 (0.61-1.74)	1.48 (0.49-4.50)	1.38 (0.38-5.01)
4 th quartile	1.30 (0.82-2.04)	1.38 (0.82-2.34)	2.19 (0.80-6.03)	2.02 (0.59-6.88)
Ptrend	0.35	0.29	0.06	0.16

Model 1; hazard ratio (95% confidence interval) adjusted for age and sex

Model 2; hazard ratio (95% confidence interval) adjusted for body mass index, total cholesterol, high density lipid-cholesterol, systolic blood pressure, carotid plaques, current smoking, diabetes mellitus, white cell count and C-reactive protein

Because an association of Lp-PLA2 with stroke has been reported, and stroke is associated with an increased risk of dementia, we subsequently excluded all people who had experienced a stroke before entering our study (n=53) and the people with missing information regarding previous stroke (n=35) and censored the incident stroke cases (n=103). This only marginally changed the effect estimates; if anything, the effect became stronger. Compared to the first



quartile, age- and sex adjusted hazard ratios of the second, third and fourth quartile were 1.16 (95% CI 0.74-1.82), 1.15 (95% CI 0.71-1.87) and 1.64 (95% CI 1.06-2.54), p-value for trend 0.03. After additional adjustment these estimates were 1.20 (95% CI 0.75-1.91), 1.23 (95% CI 0.74-2.05) and 1.80 (95% CI 1.08-2.99), p-value for trend 0.03. Excluding persons who used lipid-lowering medication at baseline (n=40) did not change the estimates.

Discussion

We found that higher plasma levels of Lp-PLA2 activity were associated with an increased risk of dementia. In particular, persons in the highest quartile of Lp-PLA2 levels were at higher risk of dementia. The association was independent of C-reactive protein and cardiovascular risk factors, and could not be explained by previous or incident stroke. The strengths of our study are its prospective design and the population-based setting with a large number of subjects. Furthermore, the follow-up with respect to the diagnosis of dementia was virtually complete and selection bias is unlikely. Although we adjusted for a large number of potential confounders, residual confounding could have occurred. Lp-PLA2 was measured in non-fasting blood, which may have increased variability in levels and thereby may have reduced the power in our study to find an association. Also, because no data were available on low density lipid (LDL)-cholesterol, we could only adjust for total cholesterol and HDL-cholesterol in our analyses. Lp-PLA2 is a marker of systemic inflammation. Levels in animals are substantially increased after injecting endotoxin and plasma levels are increased in a variety of inflammatory conditions.^{5,28} Furthermore, Lp-PLA2 is produced mainly by macrophages and expression is regulated by inflammatory cytokines.²⁹ To investigate whether the association between Lp-PLA2 and the risk of dementia could be explained through an inflammatory pathway, we adjusted for C-reactive protein and white cell count in our multivariate analyses. However, this did not change the estimate. This is in line with observations on cardiovascular disease and stroke, where Lp-PLA2 was found to be a risk factor independent of C-reactive protein and white cell count.^{2,8} Moreover, we found no correlation between levels of C-reactive protein and levels of Lp-PLA2, in accordance with results of previous studies.² A possible explanation for this is that C-reactive protein and Lp-PLA2 are produced in different tissues in response to different patterns of cytokines.⁷ We found only a weak correlation between levels of Lp-PLA2 and white cell count. It should be noted that there was not a compelling gradient of risk of dementia for levels of Lp-PLA2. Though we found a significant trend over the quartiles, in particular persons in the highest quartile of Lp-PLA2 were at higher risk of dementia. Possibly, a threshold effect is present, though this has not been found in relation to stroke and coronary heart disease. Also, the association between Lp-PLA2 and risk of dementia (HR (95% CI) for the upper quartile compared to the lower 1.56 (1.03-2.37)) was weaker than between Lp-PLA2 and ischemic stroke and coronary heart disease (HR (95% CI) for the upper quartile compared to the lower 1.97 (1.04-3.74) and 2.36 (1.58-3.52), respectively)⁸ in the Rotterdam Study. It is important that our findings are replicated.





There is strong evidence that inflammation is important in dementia. Elevated levels of inflammatory proteins such as alpha-1-antichymotrypsin (ACT) and IL-6 were found in plasma of patients with Alzheimer's disease years before the clinical dementia syndrome developed.⁹ Furthermore, a beneficial effect of NSAIDs has been suggested.³⁰⁻³³ How exactly peripheral inflammatory markers reflect or affect processes in the brain is not known. The notion that atherosclerosis plays a role in this, is supported by our observation that the association of high levels of Lp-PLA2 seemed stronger in vascular dementia than in Alzheimer's disease. However, the hypothesis of a vascular mechanism is not supported by the finding that adjusting for atherosclerosis measures did not change the estimates. Since we could only take into account clinical stroke, we can not rule out the possibility that the relation is mediated by subclinical vascular events such as silent brain infarcts, that are important risk factors for dementia.¹⁴ An alternative explanation would be that Lp-PLA2 levels could directly affect the brain or key molecules that are implicated in Alzheimer's disease such as amyloid and tau. However, we are not aware of such a relation. The role of cholesterol is not clear. In plasma, 80% of Lp-PLA2 circulates bound to LDL-cholesterol and HDL-cholesterol contains 15-20% of the enzyme mass or activity. The ARIC study found an independent effect of Lp-PLA2 on cardiovascular disease in subjects with LDL-cholesterol below the median only.³ The same relation was found in the WOSCOPS, a study conducted among men with elevated cholesterol levels.² In the Rotterdam Study, in men and women, the association with cardiovascular disease was present over the entire range of non-HDL-cholesterol. Looking at dementia, we did not find significant interactions of Lp-PLA2 and total cholesterol or HDL-cholesterol. After adjustment for total cholesterol and HDL-cholesterol, the association between Lp-PLA2 and dementia in women became slightly stronger whereas in men the association attenuated. We have no specific explanation for these differences. It should be noted that the role of cholesterol in dementia is far less clear than it is in cardiovascular disease. Moreover, in our data cholesterol was not associated with the risk of dementia. Our data suggest that the relation between Lp-PLA2 and the risk of dementia may be different in men and women. It is not uncommon for a risk factor to have sex specific effects.³⁴ In our study, the interaction term of sex and mean Lp-PLA2 activity was only borderline significant, though this might also be due to a lack of power. Regarding cardiovascular disease, the Women's Health Study did not find an association with Lp-PLA2 in women after adjustment for cardiovascular risk factors.³⁵ The importance of Lp-PLA2 is increasingly being recognized in cardiovascular disease and measuring plasma Lp-PLA2 levels in the clinical setting to predict the risk of coronary heart disease, together with the more standard predictors such as cholesterol, is now being considered. Our data suggest that it is also worthwhile to further investigate Lp-PLA2 as a possible novel risk factor for dementia.



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Chapter 2.2

Fibrinogen is associated with an increased risk of Alzheimer's disease and vascular dementia

Abstract

Background and purpose Vascular and inflammatory factors may play an important role in the pathogenesis of dementia. Studies reported an association between plasma levels of inflammation markers and the risk of dementia. Both fibrinogen and C-reactive protein are considered inflammatory markers. Fibrinogen also has important hemostatic properties. We investigated the association of fibrinogen and C-reactive protein with dementia.

Methods The study was based on the prospective population-based Rotterdam Study. Fibrinogen was measured in a random sample of 2,835 persons. High sensitivity C-reactive protein was measured in the total cohort of 6,713 persons. We identified 395 incident dementia cases during follow-up (mean 5.7 years). We estimated the associations of fibrinogen and C-reactive protein with dementia using Cox' proportional hazard models.

Results Persons with higher levels of fibrinogen had an increased risk of dementia. The hazard ratio for dementia per standard deviation increase of fibrinogen was 1.26 (95% confidence interval (CI) 1.11-1.44) adjusted for age and sex, and 1.30 (95% CI 1.13-1.50) after additional adjustment for cardiovascular factors and stroke. For Alzheimer's disease the adjusted hazard ratio was 1.25 (95% CI 1.04-1.49) and for vascular dementia 1.76 (95% CI 1.34-2.30). High levels of C-reactive protein were not associated with an increased risk of dementia.

Conclusions High fibrinogen levels were associated with an increased risk of both Alzheimer's disease and vascular dementia, but levels of C-reactive protein were not. This suggests that the increased risk of dementia associated with fibrinogen is due to the hemostatic rather than the inflammatory properties of fibrinogen.

Introduction

Vascular factors are believed to play an important role in the pathogenesis of dementia, both Alzheimer's disease and vascular dementia.^{1,2} Both inflammatory and hemostatic factors have been implicated in the development of vascular disease.

There is evidence for a role of inflammation in dementia. Signs of inflammation, such as activated microglia and inflammatory mediators including C-reactive protein and complement factors,³ are present in the brain of demented persons. It is thought that this inflammatory response contributes to neuronal death. Also, a beneficial effect of NSAIDs has been suggested.⁴ However, it is less clear how this inflammatory process affects, or is affected by, peripheral inflammatory disease or markers of disease. Previous studies suggested that peripheral markers of inflammation are elevated in plasma of patients years before the clinical syndrome of dementia developed.^{5,6}





Both fibrinogen and C-reactive protein are acute phase proteins. High levels serve as nonspecific markers for inflammatory disease. Fibrinogen also has important hemostatic properties as it affects platelet aggregation and endothelial function. Fibrinogen is a major determinant of plasma viscosity and induces red cell aggregation. High levels of fibrinogen in plasma might reduce blood flow, predispose to thrombosis and enhance atherogenesis.⁷ High levels of fibrinogen and C-reactive protein are associated with an increased risk of cardiovascular disease and stroke.⁷⁻⁹ Whether increased levels reflect active involvement in the pathogenesis of atherosclerosis or merely reflect the presence of nonspecific inflammatory disease is not clear. Since dementia is associated with both vascular and inflammatory factors, we hypothesized a relation between both fibrinogen and C-reactive protein and dementia. We investigated this association in the Rotterdam Study, a prospective population-based cohort study among men and women aged 55 years and over.

Materials and Methods

Study population and design

The Rotterdam Study is a population-based prospective cohort study that was designed to investigate the incidence and causes of cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases in the elderly. From 1990-1993, all 10,275 residents aged 55 years or over of Ommoord, a district of the city of Rotterdam, were invited to participate and 7,983 (78%) men and women agreed. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. At the baseline clinical examination, 7,047 persons were screened for dementia and had blood samples drawn. Prevalent dementia was diagnosed in 334 persons, resulting in a cohort of 6,713 persons at risk for dementia. Follow-up examinations were conducted in 1993-1994 and in 1997-1999. In addition, through linkage with records of general practitioners, the total cohort was continuously monitored for morbidity and mortality. This resulted in a virtually complete follow-up until January 1st 2000.

Measurement of fibrinogen and C-reactive protein

Platelet poor plasma was frozen in liquid nitrogen and stored at -80°C until determination. Fibrinogen measurements were done at baseline in a random sample, and were available for 2,835 of the persons at risk. Fibrinogen levels were derived from the clotting curve of the prothrombin time assay using Thromborel S as a reagent on an Automated Coagulation Laboratory (ACL 300, Instrumentation Laboratory). The coefficient of variation was 5%. High sensitivity C-reactive protein (HsCRP) was measured for the total cohort in baseline serum samples kept frozen at -20°C , using a rate near infrared particle immunoassay (NIPIA) method (Immage[®], Beckman Coulter). The range of measurement was 0.2-1440 mg/L with a variation coefficient of 3.1%. In a random sample of the study (n=29), we compared HsCRP





measurements from baseline blood stored at -20°C and -80°C . The correlation between the measurements was high (Spearman's correlation 0.99, $p\text{-value} < 0.001$). HsCRP levels were somewhat lower in blood stored at -20°C (mean difference 95% confidence interval (CI) -0.5097 ($-1.637; 0.618$)). Since the lowering of HsCRP levels was proportional we do not expect it to affect the estimate.

Diagnosis of dementia

The diagnosis of dementia was made following a three-step protocol. Two brief tests of cognition (Mini-Mental State Examination (MMSE)¹⁰ and Geriatric Mental State schedule (GMS)¹¹ organic level were used to screen all subjects. Screen-positives (MMSE score < 26 or GMS organic level > 0) underwent the Cambridge examination for mental disorders of the elderly (Camdex).¹² Subjects who were suspected of having dementia were, if necessary, examined by a neuropsychologist. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. The diagnoses of dementia and Alzheimer's disease were made in accordance with internationally accepted criteria for dementia (DSM-III-R),¹³ Alzheimer's disease (NINCDS-ADRDA),¹⁴ and vascular dementia (NINDS-AIREN)¹⁵ by a panel of a neurologist, neuropsychologist and research physician.

Covariates

At baseline, trained investigators interviewed all participants at home, collecting information on current health status and medical history. Additionally, at the research center, clinical measures were obtained. The body mass index (BMI) was calculated (weight (kg)/length (m^2)). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. Non-fasting blood samples were drawn and immediately frozen. Total cholesterol, high density lipid (HDL)-cholesterol and glucose were measured within 2 weeks. Immediately after blood sampling, white blood cell count was assessed in citrate plasma using a Coulter Counter T540[®] (Coulter electronics, Luton, England). Quality of assessments was continuously monitored by Instruchemi[®] (Hilversum, the Netherlands). Genotyping for APOE was performed on coded DNA specimens without knowledge of diagnosis. Persons were categorized on the basis of presence or absence of an APOE $\epsilon 4$ allele. Furthermore, ultrasonography of both carotid arteries was performed. As an indicator of atherosclerosis of the carotid arteries we used intima-media thickness (IMT) and presence of carotid plaques. Common carotid IMT was determined as the average of the maximum IMT of near-and far-wall measurements, and the average of left and right common carotid IMT was computed.¹⁶ Carotid plaques were determined at six different locations: common carotid artery, carotid bifurcation, and internal carotid artery





at both left and right side.¹⁶ To assess presence of atherosclerosis of the lower extremities we obtained the ankle-to-brachial index by computing the ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm. We defined diabetes mellitus as a random or postload glucose level ≥ 11.1 mmol/l or the use of blood glucose lowering medication.

Data analysis

Fibrinogen was normally distributed and we examined the association of fibrinogen and the risk of dementia and subtypes of dementia, using Cox' proportional hazard models. First, we entered fibrinogen as a linear term (per standard deviation (SD)) in the model. Next, quintiles of fibrinogen were made and the lowest quintile was used as the reference category. Since both dementia and fibrinogen are associated with age, and cardiovascular and inflammatory factors, we adjusted for age (and sex), and additionally for cardiovascular risk factors including smoking, BMI, presence of diabetes mellitus, systolic and diastolic blood pressure, total cholesterol, HDL cholesterol and measures of atherosclerosis. Other potential confounders that we considered were presence of an APOE $\epsilon 4$ allele, stroke and the inflammatory markers white blood cell count and HsCRP.

To examine the influence of atherosclerosis, we constructed a composite measure of atherosclerosis.¹⁷ A point was added to the atherosclerosis score if the following characteristics were present: plaques in at least three locations of carotid arteries, average wall-thickness of common carotid arteries in the highest quintile of the distribution, and evidence of peripheral arterial disease, defined as the ankle-brachial index less than 0.90. The atherosclerosis sum score was analyzed in four categories corresponding to score values of 0-3.

To assess whether the effect of inflammatory markers was different in people with and without previous stroke, we performed stratified analysis. We repeated the analyses excluding previous stroke and censoring incident stroke cases.

Because the distribution of HsCRP levels was skewed, we used log-transformed HsCRP in the analyses. Extreme high values may indicate the presence of an active inflammatory disease. Therefore, we excluded persons with HsCRP levels above three times the standard deviation of the log-transformed HsCRP, resulting in a total of 6,247 measurements. We examined the association between logtransformed HsCRP and dementia entering C-reactive protein as a linear term (per SD) in the model. Then, quintiles were made and the lowest quintile was used as the reference category. Adjustments were made for cardiovascular risk factors, presence of APOE $\epsilon 4$, previous stroke, white blood cell count and fibrinogen.





Results

In the total cohort (random sample) we identified 349 (192) patients with incident dementia, of whom 230 (124) patients were diagnosed with Alzheimer's disease, 26 (16) patients with Alzheimer's disease and cerebrovascular disease and 52 (31) patients with vascular dementia. Seventeen (7) patients developed dementia in Parkinson's disease and 24 (14) patients had dementia due to other causes such as multisystem atrophy, frontotemporal dementia and Lewy body dementia.

Table 1 shows that the cohort with fibrinogen measurements (n=2,835) was a random cohort compared to the total cohort at risk (n=6,713).

Table 1. Characteristics of random cohort (n=2,835) and total cohort at risk (n=6,713)

	Total cohort at risk	Random cohort
Age (years)(SD)	69.5 (9.1)	70.3 (8.6)
Women (%)	60	63
Systolic blood pressure (mmHg)(SD)	139.3 (22.3)	138.2 (21.3)
Total cholesterol (mmol/l)(SD)	6.6 (1.2)	6.7 (1.2)
Diabetes mellitus (%)	10	11
C-reactive protein (mg/l)†	1.9 (0.9-3.6)	1.8 (0.9-3.6)
Intima-media thickness (mm)(SD)	0.8 (0.2)	0.8 (0.2)
Carotis plaques (1-6 locations)(SD)	1.5 (1.7)	1.5 (1.7)
Ankle brachial index (SD)	1.1 (0.2)	1.1 (0.2)
Current smokers (%)	23	23
Presence of APOE ε4 allele (%)	25	26
White blood cell count (10 ⁹ /l)(SD)	6.7 (2.1)	7.0 (2.3)
Fibrinogen (g/l)(SD)	-	2.8 (0.7)

† Because of the skewed distribution of C-reactive protein, instead of the mean (SD), the median (interquartile range) is given.



Table 2. The association between fibrinogen and risk of dementia. Hazard ratio (95% confidence interval) for dementia (192 cases)

Fibrinogen (g/l)	Model 1*	Model 2†	Model 3‡
Per SD increase fibrinogen	1.26 (1.11-1.44)	1.30 (1.13-1.50)	1.23 (1.03-1.46)
1 st quintile	1 (ref)	1 (ref)	1 (ref)
2 nd quintile	1.42 (0.81-2.48)	1.50 (0.83-2.73)	1.44 (0.80-2.60)
3 rd quintile	1.29 (0.74-2.22)	1.31 (0.72-2.37)	1.21 (0.67-2.16)
4 th quintile	1.10 (0.63-1.92)	0.95 (0.51-1.76)	1.04 (0.58-1.87)
5 th quintile	1.92 (1.15-3.21)	2.09 (1.19-3.68)	1.67 (0.95-2.91)
p-trend	0.03	0.05	0.05

* adjusted for age and sex

† adjusted for age, sex, current smoking, presence of APOE ε4 allele, body mass index, presence of diabetes mellitus, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL cholesterol, atherosclerosis sum score and previous stroke

‡ adjusted for age, sex, C-reactive protein and white blood cell count

Table 2 shows that increasing levels of fibrinogen were associated with an increased risk of dementia. The association could not be explained by cardiovascular or inflammatory factors. Table 3 shows that higher levels of fibrinogen were associated with an increased risk of both Alzheimer's disease and vascular dementia, though the association was stronger with vascular dementia. The hazard ratio (95% CI) adjusted for age and sex for dementia per standard deviation increase of fibrinogen was 3.26 (1.68-6.35) for people with previous stroke (n=91) and 1.21 (1.05-1.38) in those without previous stroke (n=2,808). The p-value of the interaction term of fibrinogen and previous stroke was <0.001. Excluding previous stroke cases (n=91) and censoring incident stroke cases (n=13) at time of stroke did not change the estimates.

Higher levels of logtransformed HsCRP were not associated with an increased risk of dementia in our study (table 4). We investigated whether the association was present in the random subset with fibrinogen measurements. The hazard ratio (95% CI) per SD increase of logtransformed HsCRP was similar (0.95 (0.81-1.11)). For Alzheimer's disease and vascular dementia the hazard ratios (95% CI) per SD increase of logtransformed HsCRP were 0.97 (95% CI 0.84-1.11) and 1.15 (95% CI 0.86-1.54), respectively. The association of fibrinogen and C-reactive protein with dementia was not different in carriers and non-carriers of the APOE ε4 allele.

Table 3. Hazard ratio (95% confidence interval) for Alzheimer's disease (124 cases) and vascular dementia (31 cases)

Fibrinogen (g/l)	Alzheimer's disease			Vascular dementia		
	Model 1*	Model 2†	Model 3‡	Model 1*	Model 2†	Model 3‡
Per SD increase	1.23 (1.04-1.46)	1.25 (1.04-1.49)	1.22 (0.98-1.52)	1.59 (1.25-2.01)	1.76 (1.34-2.30)	1.41 (0.96-2.06)
1 st quintile	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)	1(ref)
2 nd quintile	1.46 (0.73-2.91)	1.60 (0.77-3.35)	1.55 (0.73-3.28)	1.73 (0.16-19.14)	1.39 (0.12-15.74)	1.62 (0.15-17.90)
3 rd quintile	1.38 (0.71-2.70)	1.55 (0.75-3.20)	1.31 (0.62-2.74)	3.32 (0.39-28.71)	2.48 (0.27-22.89)	2.97 (0.34-25.75)
4 th quintile	1.27 (0.65-2.49)	1.16 (0.55-2.45)	1.30 (0.63-2.71)	3.82 (0.46-32.02)	2.62 (0.28-24.34)	2.81 (0.32-24.48)
5 th quintile	1.75 (0.92-3.33)	1.95 (0.96-4.00)	1.67 (0.80-3.47)	9.50 (1.24-73.13)	8.68 (1.08-69.71)	5.39 (0.66-44.27)
p-trend	0.17	0.20	0.32	0.001	0.002	0.04

* adjusted for age and sex

† adjusted for age, sex, current smoking, presence of APOE ε4 allele, body mass index, presence of diabetes mellitus, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL cholesterol, atherosclerosis sum score and previous stroke

‡ adjusted for age, sex, C-reactive protein and white blood cell count



Table 4. The association between C-reactive protein and risk of dementia. Hazard ratio (95% confidence interval) for dementia

CRP (mg/l)	Model 1*	Model 2†	Model 3‡
Per SD increase ln(CRP)	0.95 (0.84-1.06)	1.00 (0.88-1.14)	0.91 (0.81-1.03)
1 st quintile	1(ref)	1(ref)	1(ref)
2 nd quintile	0.79 (0.56-1.11)	0.81 (0.55-1.20)	0.71 (0.50-1.02)
3 rd quintile	0.65 (0.46-0.93)	0.72 (0.49-1.07)	0.61 (0.42-0.88)
4 th quintile	0.63 (0.44-0.89)	0.68 (0.45-1.00)	0.57 (0.40-0.83)
5 th quintile	0.99 (0.72-1.37)	1.17 (0.81-1.71)	0.90 (0.64-1.26)
p-trend	0.64	0.74	0.36

N= 6,247 (measurements exceeding three times the standard deviation have been excluded from the analyses)

* adjusted for age and sex

† adjusted for age, sex, current smoking, presence of APOE ε4 allele, body mass index, presence of diabetes mellitus, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL cholesterol, atherosclerosis sum score and previous stroke

‡ adjusted for age, sex, fibrinogen and white blood cell count

Discussion

We found that higher levels of fibrinogen but not of HsCRP were associated with an increased risk of both vascular dementia and Alzheimer's disease. This association was independent of cardiovascular risk factors and other inflammatory markers, such as white blood cell count. The occurrence of clinical stroke could not explain this association.

The strengths of the Rotterdam study are its prospective design, the population-based setting and its large number of subjects. Since follow-up with respect to the diagnosis of dementia was virtually complete, selection bias is unlikely. Unfortunately, no data were available on other indicators of the coagulation and fibrinolytic system. It is difficult to differentiate between vascular dementia and Alzheimer's disease and some misclassification could have occurred classifying these subtypes.

An association between HsCRP level and the risk of dementia more than 20 years later has been reported in a nested case control study in the Honolulu-Asia Aging Study, a study of Japanese American men followed for several decades. A 3-fold significantly increased risk of dementia, both Alzheimer's disease and vascular dementia, was found in men in the upper three quartiles of HsCRP compared to men in the lowest quartile.⁵ The differences





in study population might explain the different results, though; in our study we did not find differences in the association between men and women. Also, it is possible that midlife HsCRP is associated with risk of late life dementia, as seems to be the case for more cardiovascular risk factors such as cholesterol and high blood pressure. A case-cohort study within the Rotterdam study showed that high levels of inflammatory proteins alpha1-antichymotrypsin and interleukin 6 were associated with an increased risk of dementia. Elevated levels were present years before onset of clinical disease.⁶ In this study, the association between HsCRP and dementia was weak and not significant (hazard ratio (95% CI) 1.12 (0.99-1.25)). Our findings regarding HsCRP in the present study do not contradict this observation. Alpha-antichymotrypsin is known to reinforce the formation of β -amyloid deposits and could thereby directly affect development of dementia.

We could not confirm a positive association between HsCRP and risk of dementia. Both fibrinogen and HsCRP are acute phase proteins and high levels serve as non-specific markers for inflammatory disease. Correlation between these two markers is high (0.40 with a p-value of <0.001). Since we found that fibrinogen was independently associated with risk of dementia whereas HsCRP was not, other properties of fibrinogen play a role. Fibrinogen is an inflammatory marker as well as an important factor in the coagulation cascade. It is also a major determinant of plasma viscosity and affects endothelial function.⁷ Hyperfibrinogenemia may lead to reduced blood flow and enhanced thrombosis. These hemostatic properties can explain the association with dementia. We cannot exclude that increased fibrinogen levels occur as an epiphenomenon of dementia-related processes and are not causally related. However, there is mounting evidence that vascular factors play a role in cognitive decline and dementia, both the vascular dementia and the Alzheimer's disease subtype.

The role of plasma fibrinogen in the pathogenesis of dementia is not known. In a cross-sectional study, Stott et al showed raised levels of plasma fibrinogen in patients with ischaemic stroke and vascular dementia.¹⁸ Other case-control studies did not find significant differences in levels of fibrinogen between patients with vascular dementia or Alzheimer's disease and controls.^{19, 20} To our knowledge, the association of fibrinogen and vascular dementia and Alzheimer's disease has not been studied in a large prospective population-based setting. High levels of fibrinogen are associated with cerebrovascular disease, which may explain the association with dementia. Since adjustment and censoring for previous or incident stroke did not change the association, other mechanisms might be suggested. Possibly small vessel disease (white matter lesions) or silent cerebral infarction mediates the association. Silent brain infarcts are common in an elderly population and are associated with the risk of dementia.²¹ In patients with symptomatic small vessel disease, fibrinogen has been correlated with the amount of leukoaraiosis.²² Also, significantly higher levels of fibrinogen in plasma have been found in patients with silent (lacunar) infarction.²³ In the Austrian stroke prevention study, higher serum fibrinogen levels were independently associated with white matter intensities and lacunar lesions on MRI.²⁴ Since we do not have imaging in this population we were unable to assess this.





We found a strong association with vascular dementia (notwithstanding the small number of incident cases (n=31)), which supports the hypothesis of a vascular mechanism. If fibrinogen plays a causal role in both vascular dementia and Alzheimer's disease through vascular disease, then new perspectives regarding treatment emerge. Modifying components of coagulation and blood viscosity, such as fibrinogen, could be beneficial in prevention and control of dementia.

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Chapter 2.3

Polymorphisms and haplotypes in the C-reactive protein gene and risk of dementia

Abstract

Objective Inflammation plays a role in the pathogenesis of dementia and Alzheimer's disease (AD). Studies examining serum levels of C-reactive protein in relation to dementia yielded conflicting results. Since serum levels of C-reactive protein are partly determined by genetic factors, we examined the association between genetic variation in the C-reactive protein gene with dementia and AD.

Methods This study was performed in the Rotterdam Study, a population-based prospective cohort study among elderly. Polymorphisms in the C-reactive protein gene (1184 C>T, 2042 C>T and 2911 C>G) tagging the common haplotypes were genotyped and haplotypes were constructed. During follow-up (mean 9.2 years) 607 dementia cases were identified. We estimated the association between polymorphisms and haplotypes with dementia and AD with Cox' proportional hazard models.

Results The T allele of the C-reactive protein 2042 C>T polymorphism, related to lower serum levels of C-reactive protein, was associated with a lower risk of dementia and AD. This association was strongest in APOE $\epsilon 4$ allele carriers.

Conclusion These findings suggest that C-reactive protein plays a role in development of dementia.

Introduction

Inflammatory mechanisms are thought to play a role in the pathogenesis of dementia and Alzheimer's disease (AD). Signs of inflammation, such as activated microglia and inflammatory mediators including C-reactive protein (CRP) and complement factors, are present in brains of demented persons.² CRP is produced by neurons and has been found in association with plaques and neurofibrillary tangles.²⁴ Also, elevated tissue levels of CRP have been found in the temporal cortex of dementia patients.¹⁷

It is not clear whether the CRP level in serum is associated with dementia. In a nested case control study in the Honolulu-Asia Aging Study high sensitivity CRP (hsCRP) levels, measured in blood taken in midlife, were associated with an increased risk of dementia more than 20 years later.²⁰ However, in the Rotterdam Study we did not find an association between high levels of hsCRP measured in blood taken in late life and the risk of dementia.²³ A possible explanation for these findings is that late life CRP levels reflect lifetime cumulative exposure less well.

Serum CRP levels are determined by both environmental and genetic factors.⁷ Genetic factors influencing serum CRP levels might better reflect lifelong exposure to CRP. Studying





polymorphisms in the CRP gene might therefore provide insight in the role of CRP in the etiology of dementia.

The objective of the present study was to examine whether total common genetic variation in the CRP gene was associated with the risk of dementia. We used three haplotype-tagging polymorphisms (1184 C>T (rs1130864), 2042 C>T (rs1205) and 2911 C>G (rs3093068)) to construct all common haplotypes. Previous studies showed that individual polymorphisms (2042 C>T and 1184 C>T) and constructed haplotypes¹² were associated with serum CRP levels. We examined the association of individual polymorphisms and haplotypes with dementia and AD in the Rotterdam Study, a prospective population-based cohort study among elderly.

Subjects and methods

The Rotterdam Study is a population-based prospective cohort study that investigates the incidence and risk factors of cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases in the elderly.¹⁰ From 1990-1993, all 10,275 residents aged 55 years or over of Ommoord, a district of the city of Rotterdam, were invited to participate in an extensive home interview and two visits to the research center, and 7,983 (78%) of them agreed. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. Follow-up examinations were conducted in 1993-1994, 1997-1999 and 2002-2004. In addition, through linkage with records of general practitioners, the total cohort was continuously monitored for morbidity and mortality. This resulted in a virtually complete follow-up until January 1, 2005. The diagnosis of dementia was made following a three-step protocol.^{3,16} Two brief tests of cognition (Mini-Mental State Examination (MMSE)⁹ and Geriatric Mental State schedule (GMS)⁶ organic level) were used to screen all subjects. Screen-positives (MMSE score <26 or GMS organic level >0) underwent the Cambridge examination for mental disorders of the elderly (Camdex).¹⁹ Persons who were suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for diagnosis. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Healthcare. The diagnosis of dementia and subtype of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III),¹ Alzheimer's disease (NINCDS-ADRDA),¹⁴ and vascular dementia (NINDS-AIREN)¹⁸ by a panel of a neurologist, neuropsychologist and research physician.





Single nucleotide polymorphisms (SNPs) selection and genotyping

The Seattle SNPs program for Genomic Applications has identified 31 SNPs in the CRP gene and has established that, based on SNPs with overall frequencies above 5%, four CRP gene haplotypes are present in 23 unrelated individuals of European descent from the CEPH pedigrees (<http://pga.gs.washington.edu/data/crp>, “visual haplotype” option). The haplotype block structure shows that 3 SNPs tag all four common haplotypes that describe the total common variation across the CRP gene, including 1500 bp in the promoter to 3500 bp in the 3' UTR. These three tagging SNPs were chosen partly based on their presence in existing literature and on their location in the CRP gene.

The 1184 C>T (also referred to as 3014 C>T or 1444 C>T, rs1130864), 2042 C>T (also referred to as 3872 C>T or 1846 C>T, rs1205) and the 2911 C>G (also referred to as 4741 C>G, rs3093068) polymorphisms that tag haplotypes describing the total common variation in the CRP gene were genotyped. The polymorphisms are described in relation to the start of the coding sequence of exon 1 using the Human May 2004 (hg 17) assembly (<http://genome.ucsc.edu>). These polymorphisms have also been described at <http://www.ncbi.nlm.nih.gov/SNP>.

Genotyping was performed using baseline blood samples stored at -80°C . DNA was isolated using standard procedures. Genotypes were determined in 2-ng genomic DNA with the Taqman allelic discrimination assay (Applied Biosystems, Foster City, California) using the SNP assay-by-design service of Applied Biosystems. Reactions were performed with the Taqman Prism 7900HT 384 wells format.

Haplotypes were estimated with the PHASE program²² which implements a Bayesian statistical method for reconstructing haplotypes from population genotype data. Each of the three determined CRP SNPs tagged a specific common haplotype, and the remaining fourth common haplotype could be inferred from all three SNPs.

This resulted in four most common haplotypes in our population. Haplotype alleles were coded as haplotype numbers 1 through 4 in order of decreasing frequency in the population (coding from 1184 C>T, 2042 C>T and 2911 C>G, haplotype 1=C-T-C, 2= T-C-C, 3= C-C-C, and 4=C-C-G). Haplotype 1 was defined by the rare allele of 2042 C>T, haplotype 2 by the rare allele of 1184 C>T, haplotype 3 by the absence of rare alleles of all three polymorphisms, and haplotype 4 by the rare allele of 2911 C>G.

Previously, we found associations between individual tagging polymorphisms and haplotypes with serum hsCRP levels in the Rotterdam Study. The 1184 T and 2911 G alleles were associated with higher and the 2042 T allele with lower serum hsCRP levels compared to the wildtype. Compared to haplotype 1, haplotypes 2, 3, and 4 were associated with higher levels of serum hsCRP.¹²





Covariates

Genotyping for APOE was performed on coded DNA specimens without knowledge of diagnosis. A polymerase chain reaction was performed. Persons were categorized on the basis of presence or absence of an APOE $\epsilon 4$ allele.

CRP was measured in baseline serum samples kept frozen at -20°C , using a high sensitivity rate near infrared particle immunoassay (NIPIA) method (Image[®], Beckman Coulter).

The range of measurement was 0.2-1440 mg/L with a variation coefficient of 3.1%.

Ultrasonography of both carotid arteries was performed. As an indicator of atherosclerosis of the carotid arteries we used intima-media thickness (IMT). Common carotid IMT was determined as the average of the maximum IMT of near-and far-wall measurements, and the average of left and right common carotid IMT was computed.

Data analysis

The cohort at risk consisted of 7,046 persons who were cognitively screened and not demented at baseline examination. In this cohort data on genetic variation were available in 5,972 persons.

First, to take into account phase ambiguity of the estimated haplotypes, we used the method of weighted Cox proportional hazard regression to estimate haplotype effects, based on the method of Souverein et al. This method incorporates the uncertainty about phase ambiguous individuals as weights in the model.²¹

Next, since in our population haplotype alleles could be inferred with 99% to 100% certainty in over 99% of the cases, we decided it was justified to treat the estimated haplotypes as if they were observed and used standard statistical methods in the analyses. We preferred this method because effects in individuals are estimated as opposed to the allele-based method. We assessed the relation between genotypes and dementia with Cox' proportional hazard models, adjusted for age and sex. Follow-up time in the analyses was defined as time to date of diagnosis of dementia, time to date of death or time to end of study. In the polymorphism analyses, persons homozygous for the common allele were used as the reference category. In the haplotype analyses we used persons with haplotype 1 on both alleles as the reference category because the 1-1 haplotype was associated with the lowest levels of hsCRP. We examined the association between CRP polymorphisms and dementia in carriers and non-carriers of the APOE $\epsilon 4$ allele and computed interaction terms between CRP polymorphisms and APOE genotype. Interaction terms between polymorphisms and APOE genotype were computed by multiplying the variable that encodes different genotypes of that specific CRP polymorphism with the variable that encodes presence or absence of the APOE $\epsilon 4$ allele. To examine the effect of atherosclerosis in the relation between CRP haplotypes and risk of dementia, we repeated the analyses additionally adjusting for carotid IMT, an indicator of atherosclerosis.





Results

During a mean (SD) follow-up of 9.2 (3.2) years, we identified 607 persons in the cohort at risk with incident dementia, of whom 475 were diagnosed with Alzheimer's disease. Sixty-six patients with vascular dementia were identified, 28 patients with dementia in Parkinson's disease, and 38 patients with dementia due to other causes such as multisystem atrophy, frontotemporal dementia and Lewy body dementia.

All genotype distributions in the study population were in Hardy-Weinberg equilibrium. Using the Seattle SNPs website and the HapMap website (<http://www.hapmap.org>), the SNPs were found to lie in one linkage disequilibrium block. Baseline characteristics and distribution of genotypes in the study population are shown in table 1.

Table 1. Baseline characteristics of study population (n=5972)

Age (SD) (years)		68.9 (8.7)
Sex (female) %		59
Diabetes mellitus %		10
Presence of APOE ε4 allele %		25
Body mass index (SD) (kg/m ²)		26.3 (4.0)
Intima media thickness (SD) (mm)		0.80 (0.16)
Current smoking %		23
C-reactive protein (mg/L)*		1.9 (0.9-3.6)
CRP 1184 polymorphism	CC	2743 (47.0%)
	CT	2519 (43.2%)
	TT	570 (9.8%)
CRP 2042 polymorphism	CC	2671 (44.7%)
	CT	2693 (45.1%)
	TT	603 (10.2%)
CRP 2911 polymorphism	CC	5180 (88.7%)
	CG	633 (10.8)
	GG	28 (0.5%)
	<i>Total</i>	<i>Cases/Controls</i>
Haplotype 1 (CTC=010)	3753 (32.8%)	346 (29.7%)/3407 (33.2%)
Haplotype 2 (TCC=100)	3592 (31.4%)	376 (32.2%)/3216 (31.3%)
Haplotype 3 (CCC=000)	3415 (29.9%)	377 (32.3%)/3038 (29.6%)
Haplotype 4 (CCG=001)	674 (5.9%)	67 (5.7%)/607 (5.9%)

* Because of the skewed distribution of C-reactive protein, the median (interquartile range) is given.





The T allele of the CRP 2042 C>T polymorphism was associated with a lower risk of dementia (table 2). Adjusting for hsCRP levels did not change the estimates (adjusted hazard ratios (HR) (95% confidence interval (CI)) for dementia were 0.86 (0.72-1.02) for CT and 0.72 (0.52-0.99) for TT genotypes compared to CC genotype). The CRP 1184 C>T and CRP 2911 C>G polymorphisms were not associated with risk of dementia or Alzheimer's disease.

Table 2. C-reactive protein genotypes and risk of dementia and Alzheimer's disease (hazard ratio (HR) and 95% confidence interval (CI))[¶]

		Dementia	Alzheimer's disease
		HR (95% CI)	HR (95% CI)
CRP 1184	CC	1.00 (ref)	1.00 (ref)
	CT	1.12 (0.94-1.32)	1.02 (0.85-1.24)
	TT	0.89 (0.66-1.21)	0.95 (0.68-1.32)
	Ptrend	0.89	0.92
CRP 2042	CC	1.00 (ref)	1.00 (ref)
	CT	0.84 (0.71-1.00)	0.92 (0.77-1.11)
	TT	0.73 (0.53-0.98)	0.73 (0.53-0.98)
	Ptrend	0.01	0.05
CRP 2911	CC	1.00 (ref)	1.00 (ref)
	CG	1.14 (0.89-1.47)	-
	GG	-	-
	Ptrend	0.81	-

[¶] Adjusted for age and sex

Using the weighted Cox' proportional hazard model that takes into account phase ambiguity of haplotypes, we found that haplotype 3 was associated with an increased risk of dementia compared to haplotype 1 (reference). Age and sex adjusted odds ratio (95% CI) for dementia was 1.21 (1.05-1.41). Haplotype 2 and haplotype 4 were not significantly associated with dementia (age and sex adjusted odds ratio (95% CI) 1.13 (0.97-1.31) and 1.11 (0.86-1.43)). In the tables, we present the results from the standard Cox' proportional hazard models analyses. Table 3 shows that carriers of haplotype 3 had an increased risk of dementia and Alzheimer's disease compared with the 1-1 haplotype. This finding is in accordance with the results of the polymorphism CRP 2042 C>T analysis. Table 4 shows that the association between CRP 2042 C>T and a lower risk of dementia was more pronounced in carriers of the APOE ε4 allele. Adjusting for serum hsCRP in the analyses did not affect the estimates. The



interaction term of APOE $\epsilon 4$ and CRP 2042 C>T was borderline significant (p-value 0.055). Associations of the CRP 1184 C>T and CRP 2911 C>G polymorphism with risk of dementia did not differ across carriers and non-carriers of APOE $\epsilon 4$ (data not shown).

Table 3. Haplotype combinations and risk of dementia and Alzheimer's disease (hazard ratio (HR) and 95% confidence interval (CI))[¶]

Haplotype combinations	N	Dementia HR (95% CI)	Alzheimer's disease HR (95% CI)
1-1	585	1.00 (ref)	1.00 (ref)
1-2	1200	1.19 (0.84-1.67)	1.29 (0.87-1.91)
1-3	1157	1.17 (0.83-1.66)	1.34 (0.90-1.98)
1-4	226	1.27 (0.78-2.08)	1.54 (0.89-2.65)
2-2	558	1.09 (0.73-1.63)	1.27 (0.81-2.00)
3-2	1071	1.48 (1.06-2.07)	1.42 (0.96-2.09)
3-3	497	1.44 (0.98-2.14)	1.60 (1.02-2.49)
3-4	193	1.64 (0.98-2.75)	1.54 (0.83-2.87)
4-2	205	1.41 (0.85-2.34)	1.31 (0.71-2.40)
4-4	25	-	-

[¶] Adjusted for age and sex

Additionally adjusting for carotid IMT did not change the association between CRP haplotypes and risk of dementia. Compared to the 1-1 haplotype, HR (95% CI) for dementia for haplotype 1-2 was 1.28 (0.84-1.94), for haplotype 1-3 1.20 (0.78-1.84), for haplotype 1-4 1.20 (0.65-2.22), for haplotype 2-2 1.03 (0.62-1.70), for haplotype 3-2 1.58 (1.04-2.39), for haplotype 3-3 1.47 (0.90-2.41), for haplotype 3-4 1.76 (0.94-3.29) and for haplotype 4-2 1.79 (0.99-3.22).

Discussion

In the prospective population-based Rotterdam Study, we found that the T allele of the CRP 2042 C>T (rs1205) polymorphism, that is associated with lower hsCRP serum levels, was associated with a lower risk of dementia. Haplotype analyses confirmed the association between this allele and a lower risk of dementia. This lower risk was more pronounced in carriers of an APOE $\epsilon 4$ allele. The CRP 1184 C>T (rs1130864) and the CRP 2911 C>G (rs3093068) polymorphisms were not associated with risk of dementia. Since adjustment for



Table 4. Risk of dementia in carriers and non-carriers of the APOE ε4 allele (hazard ratio with 95% confidence interval)[¶]

	Non-carriers ε4 allele (354 cases)	Carriers ε4 allele (224 cases)
CRP 2042		
CC	1.00 (ref)	1.00 (ref)
CT	0.93 (0.75-1.16)	0.77 (0.58-1.00)
TT	0.93 (0.64-1.35)	0.50 (0.29-0.88)
Ptrend	0.543	0.005
Haplotypes		
1-1	1.00 (ref)	1.00 (ref)
1-2	1.07 (0.70-1.62)	1.39 (0.76-2.56)
1-3	0.99 (0.64-1.52)	1.43 (0.78-2.63)
1-4	1.02 (0.54-1.91)	1.89 (0.84-4.28)
2-2	0.91 (0.55-1.52)	1.44 (0.72-2.88)
3-2	1.17 (0.77-1.77)	2.23 (1.24-4.03)
3-3	1.27 (0.78-2.07)	1.46 (0.69-3.09)
3-4	1.48 (0.76-2.89)	1.99 (0.86-4.63)
4-2	0.87 (0.43-1.78)	2.81 (1.24-6.35)
4-4	-	-

[¶] Adjusted for age and sex

baseline hsCRP levels did not change the association between the CRP 2042 genotype and dementia, the association appeared to be independent of baseline hsCRP levels. This is in line with our previous finding that baseline hsCRP levels are not associated with risk of dementia. The strengths of the Rotterdam Study include its prospective design, the population-based setting, its large number of subjects, and its nearly complete follow-up. A limitation of the present study is the single measurement of hsCRP that has a large biologic variability. Another limitation is that we did not have genotype data of all the persons at risk of dementia. Persons of whom we did not have genotype data were generally older and a higher percentage was female. No age and sex adjusted differences were found in incidence of dementia and cardiovascular factors such as blood pressure, cholesterol, presence of diabetes mellitus or intima media thickness. We consider it unlikely that differences have biased our results. In the Rotterdam Study, we previously did not find an association between hsCRP levels





and dementia²³ whereas in the present study we found an association between the CRP 2042 polymorphism, associated with hsCRP levels, and risk of dementia in the present study. How can we explain these seemingly discrepant findings?

A possible explanation is that late life serum hsCRP levels do not well reflect cumulative CRP exposure. In the Honolulu-Asia Aging Study, a strong association between midlife serum hsCRP and an increased risk of dementia was found.²⁰ In multivariate analyses, persons in the upper quartiles had an almost 3-fold increased risk of dementia compared with those in the first. Possibly, midlife hsCRP levels better reflect longstanding CRP levels than late life hsCRP. Since genetic determinants of CRP levels also underlie lifelong exposure level, this might explain our different results with levels and the polymorphism.

Another possibility is that the CRP polymorphism is associated with response of the immune system as well as with baseline levels. For at least one CRP polymorphism this has been investigated and confirmed. Brull et al showed that the 1184 C>T (in their paper referred to as 1444 C>T) polymorphism influences basal and stimulated CRP level.⁴ Perhaps these stimulated levels rather than baseline levels (measured in our study) mediate the association. It is possible that the 2042 polymorphism itself is not functional but that another unidentified polymorphism in strong linkage disequilibrium with CRP 2042 might be responsible for the association between the polymorphism and risk of dementia.

Finally, we cannot exclude the possibility that the association between CRP 2042 genotype and dementia is a false positive finding.

We found that APOE genotype enhanced the association of CRP 2042 genotype and risk of dementia. In line with previous reports^{11, 13} we found that APOE ε4 allele carriers had lower serum hsCRP levels when compared to non-carriers of the allele (p-value of difference <0.001). Risk reduction associated with the T allele of the CRP 2042 polymorphism, which is also associated with lower CRP levels, was most pronounced in carriers of the APOE ε4 allele. Few studies have used our approach of describing the total common variation of the CRP gene.^{5, 12, 15} In the studies by Miller et al and Carlson et al more tagging SNPs were used than in our study to describe common variation in the CRP gene. In these studies, however, ethnic diverse populations were used. For example, the population used in the study by Carlson et al was partly of European descent and partly of African descent. Therefore, more and other haplotypes were present compared with the European population used in our study. Both Miller et al. and Carlson et al. report associations between haplotypes and serum CRP levels that are in agreement with the associations between haplotypes and CRP levels in our study.¹² Studies examining the association between variation in the CRP gene and disease have so far mainly focused on cardiovascular outcomes and results have been inconclusive.

To our knowledge, only one other study examined genetic variation in the CRP gene and the risk of dementia. This study reported on the CRP 1059 G>C (rs1800947) polymorphism and found that this was not associated with Alzheimer's disease.⁸





More studies are needed to replicate our findings regarding this association and the potential gene-gene interaction with the APOE genotype.

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Chapter 2.4

Haplotypes in the fibrinogen gene and cerebral small vessel disease. The Rotterdam Scan Study

Abstract

Objective Fibrinogen levels and fibrinogen clot structure have been implicated in pathogenesis of vascular disease. We examined fibrinogen levels and variation in fibrinogen genes (fibrinogen γ (FGG), α (FGA) and β (FGB)), associated with fibrin clot structure and fibrinogen levels, in relation to cerebral small vessel disease (SVD).

Methods and Results This study was performed in the Rotterdam Scan Study, a population-based study among 1077 elderly with cerebral MRI at baseline (1995-1996). Fibrinogen levels and haplotypes were determined in baseline blood. We examined the association of fibrinogen levels and haplotypes with silent brain infarcts and white matter lesions by means of logistic regression models.

We constructed seven haplotypes (frequency >0.01) that describe the total common variation in the FGG and FGA genes. Haplotype 2 (G-A-T-A-G-T-G) was associated with presence of silent brain infarcts when compared to the most frequent haplotype (G-G-T-G-G-T-A) (odds ratio (OR) 1.41, 95% CI 1.03-1.94). Haplotype 3 (G-G-C-G-A-T-A) was associated with periventricular white matter lesions in the highest tertile of the distribution (OR 1.40, 95% CI 1.01-1.92). No association was found between plasma fibrinogen levels and SVD.

Conclusions These findings suggest that structure of the fibrin clot may play a role in the pathogenesis of cerebral SVD rather than plasma fibrinogen levels.

Introduction

Silent brain infarcts and cerebral white matter lesions are commonly detected on brain imaging in the elderly. Both result from small vessel disease (SVD) and have been associated with an increased risk of stroke and dementia.¹ The pathogenesis of SVD is incompletely understood. Established risk factors are age and hypertension, though inflammatory, hemostatic and endothelial factors have also been implicated in the development of SVD.^{2,3} Fibrinogen has both inflammatory and hemostatic properties and higher plasma levels have been associated with increased risk of coronary artery disease, ischemic stroke and dementia.^{4,5} Also, there is increasing evidence that altered structure of the fibrin clot may be involved in the pathogenesis of atherosclerosis and thrombotic disease.⁶ Genetic and environmental influences contribute to the variation in plasma fibrinogen concentration and fibrin clot structure. Fibrinogen is primarily synthesized by hepatocytes and consists of two symmetric sets of 3 chains ($A\alpha$, $B\beta$ and γ), encoded by 3 separate genes, fibrinogen α (FGA), fibrinogen β (FGB) and fibrinogen γ (FGG) clustered on chromosome 4. The FGB gene is thought to be involved in determining fibrinogen plasma levels while the FGA and FGG genes are believed





to play a role in regulating fibrin clot structure.⁷

We hypothesized a role for both fibrinogen plasma levels and clot structure in the pathogenesis of SVD. Therefore, we investigated the association between levels of fibrinogen in plasma and presence of SVD, including silent brain infarcts and periventricular and subcortical white matter lesions, on magnetic resonance imaging (MRI) of the brain. In addition, we investigated the association between common variations in FGG, FGA and FGB genes, which are associated with fibrin clot structure and fibrinogen levels, and SVD. We performed the study in the Rotterdam Scan Study; a population based imaging study among persons between 60 and 90 years old.

Methods

Study population

The Rotterdam Scan Study was designed to study causes and consequences of brain changes in the elderly.⁸ In 1995 to 1996, participants aged 60 to 90 were randomly selected in strata of age (5 years) and sex from two large ongoing population-based studies, the Zoetermeer Study and the Rotterdam Study.⁹ A total of 1077 non-demented elderly participated in the study (overall response 63%). The medical ethics committee of the Erasmus Medical Center approved the study and all participants gave informed consent.

Measurement of fibrinogen levels

The plasma concentration of fibrinogen was determined in citrated plasma that was stored at -80° Celcius. Prior to measurement, plasma samples were defrosted at 37° Celcius for 10 minutes. The method used was based on the Von Clauss¹⁰ clotting rate assay (Fibrinogen-C kit, Instrumentation Laboratory) and performed on an Automated Coagulation Laboratory (ACL 300, Instrumentation Laboratory).

Single nucleotide polymorphisms (SNPs) selection and genotyping

The Seattle SNPs program for Genomic Applications has identified various SNPs in the FGG and FGA genes and the common haplotypes can be identified by haplotype tagging SNPs. By genotyping seven haplotype tagging SNPs we were able to infer haplotypes and describe the common variation across the FGG and FGA genes. We genotyped in the FGG and FGA gene the FGG 5836 G>A (rs2066860), FGG 7874 G>A (rs2066861), FGG 9340 T>C (rs1049636), FGA 2224 G>A (rs2070011), FGA 3655 G>A (rs2070014), FGA 3807 T>C (rs2070016) and the FGA 6534 A>G (rs6050) polymorphisms that tag haplotypes covering the total common variation in the FGG and FGA genes. In addition, we genotyped the functional -148 C>T polymorphism (rs1800787) in the FGB gene, that has been shown to directly affect the FGB promoter activity.¹¹ All polymorphisms have been described at <http://www.ncbi.nlm.nih.gov/SNP>.





DNA was isolated using standard procedures and genotyping was performed using baseline samples stored at -80° Celcius. Genotypes were determined in 2-ng genomic DNA with the Taqman allelic discrimination assay (Applied Biosystems, Foster City, California, USA). Primer and probe sequences were designed using the SNP-assay-by design service of Applied Biosystems. Reactions were performed with the Taqman Prism 7900HT 384 wells format in 2 µL of reaction volume.

Haplotype alleles present in the population were inferred by means of the haplo.em function of the program Haplo Stats (<http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html>), which computes maximum likelihood estimates of haplotype probabilities.^{12, 13} Information on all SNPs was available in 899 persons.

Outcome measures on MRI

All participants underwent MRI of the brain in 1995 to 1996. Axial T1-, T2-weighted, and proton-density scans on 1.5 Tesla MRI scanners (for participants from Zoetermeer: MR Gyroscan, Philips, Best, the Netherlands and for participants from Rotterdam: MR VISION, Siemens, Erlangen, Germany) were made. The slice thickness was 5 or 6 mm with an interslice gap of 20%.

Infarcts were defined as focal hyper-intensities on T2-weighted images, 3 mm in size or larger. Proton-density scans were used to distinguish infarcts from dilated perivascular spaces. Lesions in the white matter also had to have corresponding prominent hypo intensities on T1-weighted images, in order to distinguish them from cerebral white matter lesions. A history of stroke and TIA was obtained by self-report, and by checking medical records in all 1077 participants. An experienced neurologist (PJK) subsequently reviewed the medical history and scans and categorized the infarcts as silent or symptomatic. Silent brain infarcts were defined as evidence of one or more infarcts on MRI, without a history of a (corresponding) stroke or TIA. Participants with both symptomatic and silent infarcts were categorized in the symptomatic infarct group.

White matter lesions were considered present if visible as hyperintense on proton-density and T2-weighted images, without prominent hypo intensity on T1-weighted scans. Two raters independently scored periventricular and subcortical located white matter lesions. Both intrareader and interreader studies (n=100) showed a good to excellent agreement ($\kappa=0.79-0.90$, $r=0.88-0.95$). A detailed description of the scoring method has been reported previously.¹⁴ Briefly, severity of periventricular white matter lesions was rated semi-quantitatively at three regions (grade 0-9). A total volume of subcortical white matter lesions was approximately based on number and size of lesions in the frontal, parietal, occipital, and temporal lobes (volume range 0-29.5 ml).





Data analysis

Because of the skewed distribution of fibrinogen levels, we used logtransformed fibrinogen in the analyses. We examined the association of levels of fibrinogen with presence of silent brain infarcts and periventricular and subcortical white matter lesions (third tertile versus the lower tertile of the distribution) by means of logistic regression models, adjusted for age and sex. We examined the associations per standard deviation (SD) increase of logtransformed fibrinogen and in quartiles using the lowest quartile as the reference category. Additionally, we adjusted for cardiovascular factors, including systolic and diastolic blood pressure, body mass index, current smoking, and carotid intima media thickness (IMT) as a measure of carotid atherosclerosis.

Hardy-Weinberg equilibrium of fibrinogen polymorphisms was tested using Chi square tests. We examined the association of the -148 C>T FGB promoter polymorphism with presence of silent brain infarcts and periventricular and subcortical white matter lesions (third tertile versus the lower tertile of the distribution) by means of logistic regression models, using the CC genotype as the reference category. To test the associations of haplotypes of the FGG and FGA genes with levels of fibrinogen, silent brain infarcts and white matter lesions, we used the program Haplo Stats (<http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html>).^{12, 13, 15} The probability for each haplotype pair in each individual was assigned and then an individual's phenotype was directly modeled as a function of each inferred haplotype pair, weighed by their estimated probability, to account for haplotype ambiguity. The association between fibrinogen haplotypes and fibrinogen levels, silent brain infarction and white matter lesions was examined by means of the haplo.glm function of Haplo Stats.¹² This approach is based on generalized linear model, and computes the regression of a trait on haplotypes and other covariates. In these analyses the most frequent haplotype was used as the reference category. We adjusted for age and sex and additionally for cardiovascular factors. Since plasma fibrinogen levels are an important determinant of clot structure we repeated the analyses adjusting for plasma fibrinogen. An interaction between fibrinogen levels and clot structure on disease risk has been suggested. Therefore, we repeated the analyses stratifying on high levels (above the median) and low levels (below the median) of fibrinogen.

Results

We identified 213 silent brain infarcts on brain MRI, most of which were lacunar infarcts (n=198). The baseline characteristics of the study population are shown in table 1. Table 2 shows the constructed haplotypes and their frequencies in our population. Levels of logtransformed fibrinogen were not associated with silent brain infarcts or white matter lesions on MRI (table 3). This did not change after additional adjustment for cardiovascular factors. All individual polymorphisms were in Hardy-Weinberg equilibrium. No significant association was found between the -148 C>T FGB polymorphism and SVD.





Table 1. Baseline characteristics of the study population

Age (years) (SD*)	72.2
Sex (female) (%)	52.0
Body mass index (kg/m ²) (SD)	26.7 (3.6)
Systolic blood pressure (mmHg) (SD)	147.3 (21.6)
Diastolic blood pressure (mmHg) (SD)	78.7 (11.7)
Intima media thickness (mm) (SD)	0.9 (0.2)
Total cholesterol (mmol/l) (SD)	5.9 (1.0)
HDL-cholesterol (mmol/l) (SD)	1.3 (0.3)
Current smokers (%)	17.0
Diabetes mellitus (%)	7.0
Fibrinogen (g/l)†	3.7 (3.2-4.4)
-148 C>T FGB (N (%))	
CC genotype	662 (65.4%)
CT genotype	303 (29.9%)
TT genotype	47 (4.6%)

* SD =standard deviation

† Due to skewed distribution, median (interquartile range) is given

Table 2. Construction of haplotypes and their frequencies

	FGG gene			FGA gene				Frequency
	5836G>A (rs2066860)	7874G>A (rs2066861)	9340T>C (rs1049636)	2224G>A (rs2070011)	3655G>A (rs2070014)	3807T>C (rs2070016)	6534A>G (rs6050)	
Haplotype 1	G	G	T	G	G	T	A	0.26
Haplotype 2	G	A	T	A	G	T	G	0.25
Haplotype 3	G	G	C	G	A	T	A	0.18
Haplotype 4	G	G	T	G	G	C	A	0.13
Haplotype 5	G	G	C	A	G	T	A	0.11
Haplotype 6	A	G	T	G	G	T	A	0.03
Haplotype 7	G	G	T	A	G	T	G	0.01





Table 3. The association of fibrinogen with silent brain infarcts, periventricular and subcortical white matter lesions. Odds ratio (OR) and 95% confidence interval (CI)¶

	Silent brain infarcts	Periventricular white matter lesions	Subcortical white matter lesions
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Log transformed fibrinogen per SD*	1.03 (0.88-1.22)	0.98 (0.84-1.14)	0.94 (0.81-1.09)
1 st quartile	1.00 (ref)	1.00 (ref)	1.00 (ref)
2 nd quartile	1.47 (0.90-2.33)	0.85 (0.55-1.33)	1.13 (0.74-1.71)
3 rd quartile	1.43 (0.89-2.31)	1.00 (0.65-1.54)	1.03 (0.68-1.57)
4 th quartile	1.07 (0.65-1.74)	0.88 (0.57-1.37)	0.83 (0.54-1.27)

* SD = standard deviation

¶ Adjusted for age and sex

Table 4. The association of haplotypes of the FGG and FGA genes with silent brain infarcts, periventricular and subcortical white matter lesions. Odds ratio (OR) and 95% confidence interval (CI)¶

	Silent brain infarcts	Periventricular white matter lesions	Subcortical white matter lesions
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Haplotype 1	1.00 (ref)	1.00 (ref)	1.00 (ref)
Haplotype 2	1.41 (1.03-1.94)	1.29 (0.96-1.72)	0.90 (0.67-1.19)
Haplotype 3	1.33 (0.93-1.89)	1.40 (1.01-1.92)	0.87 (0.64-1.20)
Haplotype 4	1.39 (0.94-2.05)	1.25 (0.87-1.79)	0.95 (0.74-1.42)
Haplotype 5	1.30 (0.85-2.00)	1.08 (0.73-1.60)	0.76 (0.52-1.12)
Haplotype 6	0.43 (0.16-1.14)	0.99 (0.49-1.99)	0.66 (0.33-1.33)
Haplotype 7	1.03 (0.28-3.76)	2.08 (0.75-5.77)	0.98 (0.35-2.77)

¶ Adjusted for age and sex

Compared to the CC genotype (reference), the age and sex adjusted odds ratio (OR) (95% confidence (CI)) for presence of silent brain infarcts for the CT genotype was 1.36 (0.96-1.92)





and 1.30 (0.61-2.78) for the TT genotype (P for trend 0.10). The age and sex adjusted OR (95% CI) for periventricular white matter lesions was 1.03 (0.75-1.42) for the CT genotype and 1.26 (0.64-2.48) for the TT genotype (P for trend 0.60). The age and sex adjusted OR (95% CI) for subcortical white matter lesions was 1.12 (0.82-1.53) for the CT genotype and 0.74 (0.36-1.50) for the TT genotype (P for trend 0.99). These estimates did not change after additional adjustments for cardiovascular factors. The -148 C>T FGB polymorphism was not associated with fibrinogen levels in our population. The age and sex adjusted mean of logtransformed fibrinogen (95% CI) was 1.33 (1.31-1.35) for the CC genotype, 1.34 (1.31-1.37) for the CT genotype and 1.37 (1.30-1.44) for the TT genotype.

Haplotype reconstruction of the FGG and FGA genes resulted in 26 haplotypes, but only seven haplotypes had a frequency of >0.01 in our population. Haplotype alleles were coded as haplotype numbers 1 through 7 in order of decreasing frequency in the population (coding from 5836 G>A, 7874 G>A, 9340 T>C, 2224 G>A, 3655 G>A, 3807 T>C, 6534 A>G, haplotype 1=G-G-T-G-G-T-A, haplotype 2=G-A-T-A-G-T-G, haplotype 3=G-G-C-G-A-T-A, haplotype 4=G-G-T-G-G-C-A, haplotype 5=G-G-C-A-G-T-A, haplotype 6=A-G-T-G-G-T-A and haplotype 7=G-G-T-A-G-T-G). Haplotype analyses were based on 899 participants who for whom information on all SNPs was available.

Compared to haplotype 1, haplotype 2 was associated with more silent brain infarcts and haplotype 3 was associated with more periventricular white matter lesions in the highest tertile of the distribution (table 4). Additional adjustment for cardiovascular factors did not markedly change the associations. If anything, they got stronger (OR (95% CI) for silent brain infarcts (haplotype 2) 1.48 (1.08-2.03) and for periventricular white matter lesions (haplotype 3) 1.42 (1.02-1.96)).

No association was found between haplotypes and plasma levels of fibrinogen and adjustment for plasma fibrinogen did not change the estimates. Stratifying on high and low plasma fibrinogen also did not change the associations.

Discussion

In this study, common variation in the FGG and FGA genes was associated with the presence of silent brain infarcts and periventricular white matter lesions on brain MRI. No associations were found between levels of fibrinogen or the -148 C>T FGB promoter polymorphism and silent brain infarcts or white matter lesions. Haplotypes of the FGG, FGA and FGB were not associated with fibrinogen levels.

Though an association between plasma fibrinogen levels and SVD has been suggested,¹⁶ we were not able to confirm this finding. In addition, no association was found between the -148 C>T FGB promoter polymorphism and SVD. This is in contrast with a previous study that reported an association between the -455 G>A FGB promoter polymorphism, which is in perfect linkage disequilibrium ($r^2=1$) with the -148 C>T polymorphism, and lacunar





infarction.¹⁷ However, no fibrinogen levels were available in this study. Several other studies focused on the possible association between the -148 C>T polymorphism and ischemic stroke, and their results were inconclusive.¹⁸⁻²⁰ It should be noted that in our population the -148 C>T FGB polymorphism was not significantly associated with fibrinogen levels. In an elderly population, effects of the -148 C>T polymorphism on fibrinogen levels may be attenuated. Recently, such a decrease in raising effect on fibrinogen levels has been shown for the -455 G>A polymorphism.²¹

The lack of an association between FGG and FGA haplotypes and plasma levels of fibrinogen is in line with previous findings.^{22,23}

To date, no study has examined common genetic variation in FGG and FGA in relation to cerebrovascular disease. However, common variation in these genes has been associated with other manifestations of vascular disease, independent of fibrinogen levels. Recently, Uitte de Willige et al. studied haplotypes that describe the common variation in the FGG, FGA and FGB gene in relation to risk of deep venous thrombosis.²² The haplotype of FGG (FGG-H2), tagged by 7874 G>A (rs2066861) and comparable to haplotype 2 in our study, was associated with an increased risk of deep venous thrombosis. A study by Mannila et al. reported an association between haplotypes containing FGG SNP 9340 T>C (rs1049636, in their study named 1299+79 T>C), that tags haplotype 3 in our study, and FGA SNP 2224 (rs2070011, in their study named -58 G>A), that tags haplotype 2 in our study, and the risk of myocardial infarction.²³ Several studies have examined individual polymorphisms in the FGG and FGA genes and vascular disease risk. The 6534 A>G (rs6050, also referred to as Thr312Ala) polymorphism in the FGA gene has been associated with venous thromboembolism, pulmonary embolism and post-stroke mortality among patients with atrial fibrillation.^{24,25} Though not associated with fibrinogen levels, there is evidence that variation in the FGA and FGG genes has functional implications. Uitte de Willige et al reported that the FGG-H2 was associated with reduced levels of fibrinogen γ' (and a reduced γ'/γ ratio), a product of alternative splicing of the FGG gene. It is not clear though how this reduced ratio influences fibrin formation and degradation. Several studies have provided evidence for a role of plasma fibrinogen γ' in disease risk.^{26,27} Recently, Mannila et al. showed that the FGA 2224G>A SNP constitutes an independent determinant of fibrin clot porosity and is involved in epistatic interactions on plasma fibrinogen concentration.²⁸

Also, evidence for a functional role of the FGA 6534 A>G SNP exists. This SNP occurs in a region important for FXIII-dependent cross-linking processes and may affect fibrin clot structure or stiffness. It is not clear how this increase in clot stiffness leads to a tendency to embolize. Perhaps, stiffer clots are more brittle, leading to an increased tendency to fragmentize under conditions of blood flow.^{7,29} Interestingly, the FGA SNP 2224 G>A in the study by Mannila and the FGG-H2 haplotype in the study by Uitte de Willige are in strong linkage disequilibrium with the 6534 A>G polymorphism.





In our study, common variation in the FGG and FGA genes was associated with silent brain infarcts and periventricular white matter lesions, but not with subcortical white matter lesions. It has previously been suggested that different pathophysiological mechanisms may underlie periventricular and subcortical white matter lesions.^{30,31} For instance, atrial fibrillation has been found to be predominantly related to periventricular white matter lesions.³⁰ A possible explanation for this may be that periventricular white matter is an arterial border zone and therefore more vulnerable while the subcortical white matter is better vascularized.³² To conclude, in this population-based imaging study, we found that common variation in the FGG and FGA genes of fibrinogen was associated with SVD on brain MRI. Since this is the first report our study needs replication. Also, more functional studies need to be performed to elucidate the mechanism through which these genes influence risk of vascular disease. Our findings suggest that mechanisms related to fibrin clot structure are involved in the pathogenesis of SVD rather than plasma concentration of fibrinogen.

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Chapter 2.5

Haplotypes in the fibrinogen gene and dementia

Brief communication

Abstract

Background Both inflammatory and hemostatic factors have been implicated in the pathogenesis of dementia. Previously we reported an association between higher plasma levels of fibrinogen and an increased risk of dementia. Whether fibrin clot structure plays a role in development of dementia is not known. We examined the association between variation in fibrinogen genes involved in fibrin clot structure (fibrinogen γ (FGG) and α (FGA)) and dementia.

Methods The study was performed within the Rotterdam Study, a prospective population-based study among 7,983 persons of 55 years and over, ongoing since 1990. Seven tagging single nucleotide polymorphisms (SNPs) in the FGG and FGA genes were determined in blood samples drawn at the baseline examination (1990-1993). Until January 1st 2005, 743 dementia patients were identified. We examined the association between constructed haplotypes and dementia by means of logistic regression models.

Results Seven haplotypes (frequency >0.01) that describe the total common variation in FGG and FGA genes were constructed. Compared with the most frequent haplotype 1 (G-G-T-G-T-A) no association was found between haplotypes and dementia or Alzheimer's disease.

Conclusions Our findings suggest that common variation in the FGG and FGA genes is not an important risk factor for dementia.

Introduction

Both inflammatory and hemostatic factors have been implicated in the pathogenesis of dementia.^{1,2} We recently reported an association between higher plasma levels of fibrinogen, which has inflammatory and hemostatic properties, and an increased risk of dementia, both Alzheimer's disease and vascular dementia.³

Fibrinogen is primarily synthesized by hepatocytes and consists of two symmetric sets of 3 chains ($A\alpha$, $B\beta$ and γ), encoded by 3 separate genes, fibrinogen α (FGA), fibrinogen β (FGB) and fibrinogen γ (FGG) clustered on chromosome 4. The FGB gene is thought to be involved in determining fibrinogen plasma levels whereas FGG and FGA genes are believed to play a role in regulating fibrin clot structure.⁴

There is evidence that altered structure of the fibrin clot may be involved in the pathogenesis of vascular disease.⁵ In a previous study performed in the Rotterdam Scan Study we found an association of haplotypes of the FGG and FGA gene with cerebral small vessel disease as assessed with brain imaging. Since vascular disease, including cerebral small vessel disease,





is associated with dementia and Alzheimer's disease we hypothesized an association between altered fibrin clot structure and dementia.

In this study, we set out to investigate whether common variation in the FGG and FGA genes that are involved in regulating clot structure is associated with an increased risk of dementia and Alzheimer's disease. The study was performed in the Rotterdam Study, a population-based prospective cohort study among persons of 55 years and over.

Methods

The Rotterdam Study is a population-based prospective cohort study among 7983 persons of 55 years and over.⁶ Baseline examinations (1990-1993) and follow-up examinations (1993-1994, 1997-1999 and 2002-2004) included physical examinations, screening and clinical work-up for dementia. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. Through linkage with records of general practitioners, the total cohort was continuously monitored for morbidity and mortality. This resulted in a virtually complete follow-up until January 1, 2005.

The Seattle SNPs program for Genomic Applications has identified various SNPs in the FGG and FGA genes and the common haplotypes can be identified by haplotype tagging SNPs. By genotyping seven haplotype tagging SNPs we were able to infer haplotypes and describe the common variation across the FGG and FGA genes. In the FGG and FGA gene the FGG 5836 G>A (rs2066860), FGG 7874 G>A (rs2066861), FGG 9340 T>C (rs1049636), FGA 2224 G>A (rs2070011), FGA 3655 G>A (rs2070014), FGA 3807 T>C (rs2070016) and the FGA 6534 A>G (rs6050) polymorphisms that tag haplotypes describing the total common variation in the FGG and FGA gene were genotyped. These polymorphisms have also been described at <http://www.ncbi.nlm.nih.gov/SNP>.

DNA was isolated using standard procedures and genotyping was performed using baseline samples stored at -80° Celcius. Genotypes were determined in 2-ng genomic DNA with the Taqman allelic discrimination assay (Applied Biosystems, Foster City, California, USA). Primer and probe sequences were optimized by using the SNP-assay-by design service of Applied Biosystems. Reactions were performed with the Taqman Prism 7900HT 384 wells format in 2 µL of reaction volume.

Haplotype alleles present in the population were inferred by means of the haplo.em function of the program Haplo Stats (http://cran.r-project.org/src/contrib/Descriptions/haplo_stats.html), which computes maximum likelihood estimates of haplotype probabilities.^{7,8}

Information on all SNPs was available in 5538 persons.

The diagnosis of dementia and subtypes of dementia was made following a three-step protocol as described previously.⁹ We used internationally accepted criteria for dementia (DSM-IIIIR),¹⁰ Alzheimer's disease (NINCDS-ADRDA),¹¹ and vascular dementia (NINDS-AIREN)¹²





Data analysis

Hardy-Weinberg equilibrium of fibrinogen polymorphisms was tested by means of Chi square tests. To test the associations of fibrinogen haplotypes with dementia and Alzheimer's disease we used the program Haplo Stats (<http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html>).^{7, 8, 13} The probability for each haplotype pair in each individual was assigned and then an individual's phenotype was directly modeled as a function of each inferred haplotype pair, weighed by their estimated probability, to account for haplotype ambiguity. The association between fibrinogen haplotypes and dementia was examined by means of the haplo.glm function of Haplo Stats. This approach is based on generalized linear model, and computes the regression of a trait on haplotypes and other covariates. In these analyses the most frequent haplotype was used as the reference category. We adjusted for age and sex in the analyses. Because of low numbers, we were not able to assess the associations with vascular dementia.

Results

In the study population at risk of dementia with complete data on haplotypes, we identified 563 patients with dementia, including 440 patients with Alzheimer's disease and 63 with vascular dementia. The baseline characteristics of the study population are shown in table 1.

Table 1. Baseline characteristics of the study population (n=5538)

Age, years (SD*)	69.0 (8.7)
Sex, female, %	58.9
Current smokers, %	23.0
Presence of an APOE ε4 allele, %	24.8
Body mass index, kg/m ² (SD)	26.4 (4.0)
Systolic blood pressure, mmHg (SD)	139.4 (22.1)
Diastolic blood pressure, mmHg (SD)	73.9 (11.4)
Total cholesterol, mmol/l (SD)	6.6 (1.2)
High density lipid (HDL) cholesterol, mmol/l (SD)	1.3 (0.4)
Diabetes mellitus, %	10.0
Intima media thickness (IMT), mm (SD)	0.79 (0.16)
Fibrinogen, g/l†	2.7 (2.3-3.2)

* SD = standard deviation

† Due to skewed distribution, median (interquartile range) is given





Table 2. Construction of haplotypes and their frequencies

	5836G>A (rs2066860)	7874G>A (rs2066861)	9340T>C (rs1049636)	2224G>A (rs2070011)	3655G>A (rs2070014)	3807T>C (rs2070016)	6534A>G (rs6050)	Frequency
Haplotype 1	G	G	T	G	G	T	A	0.26
Haplotype 2	G	A	T	A	G	T	G	0.26
Haplotype 3	G	G	C	G	A	T	A	0.17
Haplotype 4	G	G	T	G	G	C	A	0.12
Haplotype 5	G	G	C	A	G	T	A	0.12
Haplotype 6	A	G	T	G	G	T	A	0.04
Haplotype 7	G	G	T	A	G	T	G	0.01

All individual polymorphisms were in Hardy-Weinberg equilibrium. Haplotype reconstruction resulted in 24 haplotypes, but only seven had a frequency of >0.01 in our population. Haplotype alleles were coded as haplotype numbers 1 through 7 in order of decreasing frequency in the population (coding from 5836 G>A, 7874 G>A, 9340 T>C, 2224 G>A, 3655 G>A, 3807 T>C, 6534 A>G, haplotype 1=G-G-T-G-G-T-A, haplotype 2=G-A-T-A-G-T-G, haplotype 3=G-G-C-G-A-T-A, haplotype 4=G-G-T-G-G-C-A, haplotype 5=G-G-C-A-G-T-A, haplotype 6=A-G-T-G-G-T-A and haplotype 7=G-G-T-A-G-T-G). Table 2 shows the constructed haplotypes and their frequencies in our population.

We did not find associations of haplotypes with dementia or Alzheimer's disease (table 3).

Table 3. The association of haplotypes in the FGA and FGG genes with dementia and subtypes of dementia. Odds ratios (OR) and 95% confidence intervals (CI)

	OR (95% CI)* for dementia	OR (95% CI)* for Alzheimer's disease
Haplotype 1	1.00 (ref)	1.00 (ref)
Haplotype 2	0.91 (0.75-1.08)	0.86 (0.71-1.04)
Haplotype 3	0.89 (0.73-1.09)	0.83 (0.67-1.03)
Haplotype 4	0.89 (0.72-1.11)	0.93 (0.74-1.18)
Haplotype 5	0.89 (0.71-1.12)	0.84 (0.65-1.07)
Haplotype 6	1.02 (0.72-1.45)	1.11 (0.77-1.61)
Haplotype 7	1.13 (0.63-2.02)	0.89 (0.79-1.01)

* Adjusted for age and sex





Discussion

In this large population-based prospective study, we did not find an association between common variation in the FGG and FGA genes and dementia or Alzheimer's disease. Though not associated with fibrinogen plasma levels, functional implications of variation in FGG and FGA on fibrin clot structure and stability that may explain an association with vascular disease have been described.^{14, 15} Recently, several studies reported an association between common variation in these genes and vascular disease, including myocardial infarction¹⁴ and venous thrombosis.¹⁶ In the Rotterdam Scan Study, we found an association of haplotypes in the FGG and FGA gene with presence of silent brain infarcts and periventricular white matter lesions.

This is the first study to examine common genetic variation in FGG and FGA in relation to dementia. We cannot exclude that we have missed small haplotypes effects despite the large number of dementia cases in our sample. Also, the association with vascular dementia could not be studied because of low numbers.

To conclude, our findings suggest that common variation in the FGG and FGA genes is not an important risk factor for dementia.

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Chapter 2.6

Polymorphisms in the interleukin 6 and transforming growth factor β 1 gene and risk of dementia. The Rotterdam Study

Abstract

Purpose Inflammatory mechanisms are involved in the pathogenesis of dementia.

Inflammatory cytokines, including interleukin-6 (IL-6) and transforming growth factor β 1 (TGF β 1), have been found in association with Alzheimer pathology and there is evidence for direct involvement of these cytokines in formation of amyloid plaques. Polymorphisms in genes encoding for IL-6 and TGF β 1 are associated with plasma levels of IL-6 and TGF β 1. Studies examining the association between polymorphisms in these genes and dementia yielded conflicting results. The purpose of this study was to examine the association between genetic variance in IL-6 and TGF β 1 and risk of dementia.

Methods We examined this association in the Rotterdam Study, a prospective population-based cohort study in the elderly. Polymorphisms in the IL-6 (-174G>C) and TGF β 1 gene (-800G>A, -509C>T, +10T>C, +25G>C and 263C>T) were genotyped and haplotypes of the TGF β 1 gene were constructed. In a random subset IL-6 plasma levels were measured. During follow-up (mean 9.2 years), 743 dementia cases were identified. We estimated the association between individual polymorphisms and haplotypes with dementia with Cox' proportional hazard models.

Results No association was found between the -174G>C polymorphism in the IL-6 gene and risk of dementia. No association was found between polymorphisms and constructed haplotypes in the TGF β 1 gene and risk of dementia or Alzheimer's disease. No association was found between IL-6 genotype and IL-6 plasma levels in the random subset. Associations did not differ across APOE genotypes.

Our findings do not suggest involvement of genetic variance in IL-6 and TGF β 1 in the development of dementia.

Introduction

Inflammatory processes are thought to play an important role in the pathogenesis of dementia, in particular Alzheimer's disease (AD). Signs of inflammation, such as reactive astrogliosis, activated microglia and overexpression of inflammatory molecules have been found in association with Alzheimer pathology in demented persons.² Inflammatory proteins are upregulated in the brain and serum of AD patients. Several inflammatory cytokines, including interleukin 6 (IL-6) and transforming growth factor β 1 (TGF β 1), are of particular interest because they occur in amyloid plaques in the brain and may also contribute to the formation of amyloid plaques.^{19, 35}

IL-6 has been detected in amyloid plaques in brains of AD patients.¹⁹ IL-6 is also thought to





lead to the accumulation of acute phase proteins in plaques and is associated with increased amyloid precursor protein (APP) synthesis.⁸ In transgenic mice models, elevated levels of IL-6 in the central nervous system result in neuropathogenic effects and cognitive deficits.⁷ In a cross-sectional study, high IL-6 plasma levels in healthy subjects were associated with greater cognitive decline³⁴ and in a case-cohort study within the Rotterdam Study, plasma IL-6 was associated with an increased risk of dementia during follow-up.¹² It is not clear how plasma levels of IL-6 relate to the dementia disease process in the brain. The -174 polymorphism in the promoter region of the IL-6-gene has been found to influence the transcriptional regulation of IL-6 and plasma IL-6 levels,¹⁴ which makes this polymorphism potentially interesting with regard to the development of dementia. Previous studies examining the association between the -174 polymorphism and dementia have yielded conflicting results. Some found an association^{4, 13, 20, 26, 30} whereas others did not.^{6, 8, 11, 37}

TGFβ1 is believed to be an important factor in regulating inflammatory responses. It is a major factor in cellular responses to brain injury²² and may protect the brain against neuronal degeneration.²⁷ TGFβ1 is present in amyloid plaques and is overexpressed in AD brains.³³ In APP transgenic mice over expression of TGFβ1 is associated with deposition of beta-amyloid and thus may promote or initiate beta-amyloid accumulation in plaques.³⁵ In AD patients, TGFβ1 levels in cerebrospinal fluid and serum were found to be higher than in non-demented elderly controls.^{15, 19} The -509 polymorphism T allele has been associated with higher serum concentration of TGFβ1, suggesting a functional role for this polymorphism in the regulation of TGFβ1 levels.¹⁷ Mixed results have been reported on the association of different polymorphisms in the TGFβ1-gene, including the -509 polymorphism, with dementia.^{3, 21, 24} Since there is evidence for a role of both IL-6 and TGFβ1 in the pathogenesis of dementia, the objective of the present study was to determine the associations of genetic variance in the IL-6 gene (-174 polymorphism) and in the TGFβ1 gene (-800, -509, +10, +25 and 263 polymorphisms and constructed haplotypes) and the risk of dementia. We performed the study within the Rotterdam Study, a prospective population-based cohort study among 7,983 persons of 55 years and over.

Materials and methods

The Rotterdam Study is a population-based prospective cohort study that investigates the incidence and risk factors of cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases in the elderly.^{5, 18} From 1990-1993, all 10,275 residents aged 55 years or over of Ommoord, a district of the city of Rotterdam, were invited to participate in an extensive home interview and two visits to the research center, and 7,983 (78%) of them agreed. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. Follow-up examinations were conducted in 1993-1994, 1997-1999 and 2002-2004. In addition, through linkage with





records of general practitioners, the total cohort was continuously monitored for morbidity and mortality. This resulted in a virtually complete follow-up until January 1, 2005.

The diagnosis of dementia was made following a three-step protocol.²⁵ Two brief tests of cognition (Mini-Mental State Examination (MMSE)¹⁶ and Geriatric Mental State schedule (GMS)¹⁰ organic level were used to screen all subjects. Screen-positives (MMSE score <26 or GMS organic level >0) underwent the Cambridge examination for mental disorders of the elderly (Camdex).²⁹ Persons who were suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for diagnosis. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Healthcare. The diagnosis of dementia and subtype of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III),¹ Alzheimer's disease (NINCDS-ADRDA),²³ and vascular dementia (NINDS-AIREN)²⁸ by a panel of a neurologist, neuropsychologist and research physician.

The IL-6-174 G>C, TGFβ1-800 G>A, TGFβ1-509 C>T, TGFβ1+10 T>C, TGFβ1+25 G>C and TGFβ1 263 C>T polymorphisms were genotyped.

DNA was isolated using standard procedures and genotyping was performed using baseline samples stored at -80 degrees Celcius. Genotypes were determined in 2-ng genomic DNA with the Taqman allelic discrimination assay (Applied Biosystems, Foster City, California, USA). Primer and probe sequences were optimized by using the SNP-assay-by design service of Applied Biosystems. Reactions were performed with the Taqman Prism 7900HT 384 wells format in 2 μL of reaction volume. For the TGFβ1 gene haplotypes were estimated with the PHASE program.³¹ This resulted in the four most common haplotypes that describe > 90% of our population. Haplotype alleles were coded as haplotype numbers 1 through 4 in order of decreasing frequency in the population (coding from -800 G>A, -509 C>T, +10 T>C, +25 G>C and 263 C>T, haplotype 1=G-C-T-G-C, haplotype 2= G-T-C-G-C, haplotype 3= A-C-T-G-C and haplotype 4= G-C-C-C-C).

In 714 participants plasma IL-6 levels were measured. A venapuncture was performed by application of minimal stasis with a 21-gauge Butterfly needle with tube (Surflo winged infusion set, Terumo). Nonfasting blood was collected in tubes containing 0.129 mol/L sodium citrate at 4°C. Plasma was collected after centrifugation for 10 minutes at 3000 rpm. Subsequently, platelet-free plasma was obtained by centrifugation for 10 minutes at 10000 rpm, immediately frozen in liquid nitrogen, and stored at -20°C. IL-6 levels were measured by using a commercially available ELISA (Quantikine HS from R&D Systems Europe). The inter assay coefficient of variation for IL-6 was 8.7%.

The cohort at risk consisted of 7,046 persons who were cognitively screened and not demented at baseline. Of these persons, data on the IL-6-174 polymorphism were available in 6,119





persons and data on the TGFβ1-polymorphisms in 6,144 (TGFβ1-800), 6,133 (TGFβ1-509), 5,919 (TGFβ1+10 and TGFβ1+25) and 6,182 (TGFβ1 263) persons. For the TGFβ1 polymorphism and haplotype analyses, the study population consisted of 5,919 persons of whom all polymorphism data were available.

Data analysis

First, we examined the association between different genotypes of the -174 promoter polymorphism and plasma levels of IL-6 in the random subset.

Next, we assessed the relation of polymorphisms with dementia and AD with Cox' proportional hazard models, adjusted for age and sex. Follow-up time in the analyses was defined as time to date of diagnosis of dementia, time to death or time to end of study. Persons homozygous for the common allele were used as the reference category. In the haplotype analysis, we examined the association of each haplotype with dementia and AD, using persons with no copy of that specific haplotype as the reference category. We then compared persons with one and those with two copies of that specific haplotype with the reference category. We performed this analysis for all 4 haplotypes separately.

We examined the association between polymorphisms and dementia in carriers and non-carriers of the APOE ε4 allele and computed interaction terms between IL-6 and TGFβ1 polymorphisms and the APOE genotype.

Results

During follow-up up (mean (SD) 9.2 (3.2)), we identified 743 persons with dementia, of whom 583 patients were diagnosed with Alzheimer's disease and 82 patients with vascular dementia. All genotype distributions, except for the TGFβ1 263 C>T, were in Hardy Weinberg equilibrium. Baseline characteristics and distribution of genotypes in the study population are shown in table 1.

No association was found between the IL6-174 G>C polymorphism and plasma IL-6 levels in our data. We did not find an association between the IL-6 -174 G>C polymorphism and dementia or AD (table 2). Also, no associations were found between the different TGFβ1 polymorphisms and dementia or AD (table 3). The associations for IL-6 and TGFβ1 did not differ across strata of carriers and non-carriers of the APOE ε4 allele (data not shown). Haplotype analyses of the TGFβ1 polymorphisms did not show an association between any of the four examined haplotypes and risk of dementia or AD (table 4).





Table 1. Baseline characteristics of the study population (n=7,046)

Age (SD) yrs		69.5 (9.1)	
Sex (female) %		60.0	
APOE ε4 allele %		25.1	
Polymorphisms	genotype N (%)	allele N (%)	HWE p-value†
IL-6-174	GG 2198 (35.9)		>0.10
	GC 2896 (47.3)	G 7292 (59.6)	
	CC 1025 (16.8)	C 4946 (40.4)	
TGF1β-800	GG 5110 (83.2)		>0.50
	GA 991 (16.1)	G 11211 (91.2)	
	AA 43 (0.7)	A 1077 (8.8)	
TGF1β-509	CC 3085 (50.3)		>0.05
	CT 2485 (40.5)	C 8655 (70.6)	
	TT 563 (9.2)	T 3611 (29.4)	
TGF1β+10	TT 2352 (39.7)		>0.20
	TC 2730 (46.1)	T 7434 (62.8)	
	CC 837 (14.1)	C 4404 (37.2)	
TGF1β+25	GG 5059 (85.5)		>0.20
	GC 831 (14.0)	G 10949 (92.5)	
	CC 29 (0.5)	C 889 (7.5)	
TGF1β 263	CC 5952 (96.2)		<0.01
	CT 223 (3.6)	C 12127 (98.1)	
	TT 7 (0.1)	T 237 (1.9)	
Haplotypes		N (%)	
Haplotype 1 G-C-T-G-C		6358 (53.7)	
Haplotype 2 G-T-C-G-C		3234 (27.3)	
Haplotype 3 A-C-T-G-C		1045 (8.8)	
Haplotype 4 G-C-C-C-C		885 (4.4)	

† HWE =Hardy Weinberg equilibrium





Table 2. IL-6-174 G>C polymorphism and the risk of dementia and Alzheimer's disease (AD) (hazard ratio (HR) with 95 % confidence interval (CI))¶

IL-6-174	Cases/N	HR (95% CI)	AD/N	HR (95% CI)
GG	215/2198	1.00 (ref)	162/2198	1.00 (ref)
GC	291/2896	1.02 (0.85-1.21)	228/2896	1.05 (0.86-1.28)
CC	115/1025	1.08 (0.86-1.35)	93/1025	1.15 (0.89-1.48)
	Ptrend	0.73	Ptrend	0.31

¶ Adjusted for age and sex

Table 3. Polymorphisms in the TGF1 β gene and the risk of dementia and Alzheimer's disease (AD) (hazard ratio (HR) with 95 % confidence interval (CI))¶

TGF1 β		Cases/N	HR (95% CI)	AD/N	HR (95% CI)
-800	GG	490/4914	1.00 (ref)	389/4914	1.00 (ref)
	GA	112/964	1.17 (0.96-1.44)	79/964	1.04 (0.81-1.32)
	AA	6/41	1.41 (0.63-3.16)	6/41	1.76 (0.78-3.93)
	Ptrend		0.09	Ptrend	0.43
-509	CC	315/2983	1.00 (ref)	247/2983	1.00 (ref)
	CT	252/2389	1.02 (0.87-1.21)	192/2389	1.00 (0.82-1.20)
	TT	41/547	0.81 (0.58-1.12)	35/547	0.89 (0.63-1.27)
	Ptrend		0.47	Ptrend	0.65
+10	TT	255/2352	1.00 (ref)	199/2352	1.00 (ref)
	TC	288/2730	0.97 (0.82-1.15)	220/2730	0.95 (0.78-1.15)
	CC	65/837	0.77 (0.58-1.01)	55/837	0.84 (0.63-1.14)
	Ptrend		0.10	Ptrend	0.27
+25	GG	528/5059	1.00 (ref)	411/5059	1.00 (ref)
	GC	79/831	0.86 (0.68-1.08)	62/831	0.86 (0.66-1.12)
	CC	1/29	0.29 (0.04-2.05)	1/29	0.38 (0.05-2.74)
	Ptrend		0.09	Ptrend	0.17
263	CC	587/5700	1.00 (ref)	458/5700	1.00 (ref)
	CT	21/212	1.09 (0.71-1.69)	16/212	1.15 (0.70-1.89)
	TT	0/7	-	0/7	-
	Ptrend		0.59	Ptrend	0.70

¶ Adjusted for age and sex



Table 4. Haplotypes of the TGF β 1 gene and the risk of dementia and Alzheimer's disease (AD) (hazard ratio (HR) with 95 % confidence interval (CI))[¶]

		Cases/N	HR (95% CI)	AD/N	HR (95% CI)
Haplotype 1	0	113/1281	1.00 (ref)	90/1281	1.00 (ref)
	1	318/2918	1.20 (0.97-1.48)	242/2918	0.90 (0.69-1.17)
	2	177/1720	1.11 (0.88-1.41)	142/1720	1.03 (0.83-1.26)
	Ptrend		0.51	Ptrend	0.50
Haplotype 2	0	329/3155	1.00 (ref)	257/3155	1.00 (ref)
	1	246/2294	1.04 (0.88-1.23)	189/2294	1.02 (0.85-1.24)
	2	33/470	0.75 (0.52-1.07)	28/470	0.82 (0.56-1.22)
	Ptrend		0.44	Ptrend	0.62
Haplotype 3	0	490/4914	1.00 (ref)	389/4914	1.00 (ref)
	1	112/965	1.18 (0.96-1.44)	79/965	1.04 (0.81-1.32)
	2	6/40	1.41 (0.63-3.16)	6/40	1.76 (0.78-3.94)
	Ptrend		0.09	Ptrend	0.43
Haplotype 4	0	529/5062	1.00 (ref)	411/5062	1.00 (ref)
	1	78/829	0.84 (0.67-1.07)	62/829	0.86 (0.66-1.13)
	2	1/28	0.29 (0.04-2.06)	1/28	0.39 (0.05-2.75)
	Ptrend		0.08	Ptrend	0.17

[¶] Adjusted for age and sex

Discussion

In this large population-based prospective study, we did not find an association between the -174 promoter polymorphism in the IL-6 gene or genetic variance in the TGF β 1 gene with risk of dementia.

Strengths of the Rotterdam Study are its population-based setting, prospective design, large number of dementia cases and virtually complete follow-up.

Previously, we reported on the association between higher levels of IL-6 and an increased risk of dementia in a case-cohort study within the Rotterdam Study (rate ratio (95% confidence interval (CI)) for dementia 1.28 (1.06-1.55)).¹² Plasma IL-6 levels, however, were available only in a random subset of 714 participants. We did not find a significant association between the different genotypes of the -174 promoter polymorphism and levels of IL-6 in this subset. Although an effect of the -174 polymorphism on transcription and IL-6 levels has been described, the extent of the influence on plasma IL-6 levels has not been definitely established.



It is conceivable that the effect is dependent on interactions between several polymorphisms in the IL-6 gene and that the -174 polymorphism is not an optimal marker in this respect.³² Our negative findings are in line with previous studies concerning the -174 polymorphism and dementia.^{6, 8, 11, 37} Zhang et al.³⁷ also reported on a lack of association between genotype groups of the 174-G> polymorphism and Alzheimer's pathology such as amyloid load, tau load and reactive astrocytes and microglia.

No levels of TGFβ1 were available in our study; hence we were not able to examine the association between different polymorphisms and haplotypes with plasma levels of TGFβ1 in our population. The -509 polymorphism is located in a regulatory region and Grainger et al found that the -509 T allele was associated with a higher plasma concentration of TGFβ1 compared with the -509 C allele.¹⁷

One study found an increased risk of developing Alzheimer's disease for the -509 TT genotype versus -509 CC+CT genotypes of 1.7 (95% CI=1.11-2.66).²¹ The authors of this study also reported that the -509 T allele may be associated with higher transcriptional activity than the -509 C allele. However, this elevated transcription activity was only modest. Our negative findings support those of other studies investigating the -509 C>T, -800 G>A and +25G>C polymorphisms in relation to Alzheimer's disease.^{3, 24}

We cannot exclude that we have missed small effects of the inflammatory gene polymorphisms and haplotypes studied despite the large number of cases in our sample. Other inflammatory cytokines, such as IL-1, may be associated with development of dementia.³⁶

To conclude, our findings do not suggest involvement of the -174 promoter polymorphism in the IL-6 gene in the development of dementia.

Also, we did not find evidence for a role of polymorphisms (-800, -509, +10, +25 or 263) or constructed haplotypes in the TGFβ1 gene in development of dementia.

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Chapter 3. Vascular factors and dementia

Chapter 3.1

Atherosclerosis and risk of dementia

Abstract

Objective Atherosclerosis has been implicated in the development of dementia and its major subtypes Alzheimer's disease and vascular dementia. However, support for this association mainly comes from cross-sectional studies. We investigated the association of atherosclerosis with dementia and subtypes of dementia during long follow-up, with various non-invasive measures of atherosclerosis.

Methods This study was performed within the Rotterdam Study, a population-based prospective cohort study among 7,983 elderly. At baseline (1990-1993) and at the third survey (1997-1999) carotid intima media thickness, carotid plaques, and peripheral arterial disease (measured as ankle-brachial index) were measured. During follow-up (mean 9.0 years) 743 persons developed dementia. We estimated the associations of different measures of atherosclerosis with risk of dementia and subtypes of dementia by means of Cox' proportional hazard models. Analyses were repeated stratified on duration of follow-up. To evaluate competing risk of mortality we examined the association between measures of atherosclerosis and risk of dementia or mortality by combining the two in a single outcome measure.

Results We found that atherosclerosis, predominantly carotid atherosclerosis, was associated with an increased risk of dementia during short follow-up. This association attenuated with longer follow-up, likely due to the strong association between atherosclerosis and mortality. The associations did not differ across APOE genotypes.

Interpretation Our findings suggest that atherosclerosis is associated with an increased risk of dementia. Stronger associations between atherosclerosis and mortality may attenuate the association between atherosclerosis and dementia in prospective cohort studies with long follow-up.

Introduction

Atherosclerosis is believed to be involved in development of dementia and its major subtypes vascular dementia and Alzheimer's disease.¹ The association may be mediated by cerebrovascular disease, such as stroke and cerebral small vessel disease,² or result from brain hypoperfusion.³ However, published evidence for a relation between atherosclerosis and dementia risk is scarce and the notion is mainly based on a cross-sectional report from the Rotterdam Study from 1997.⁴ In that study, we found a strong association between indicators of atherosclerosis, including vessel wall thickness and plaques of the carotid arteries,





peripheral arterial disease and a constructed atherosclerosis sumscore, and the presence of dementia. To date, one prospective study have provided evidence for a role of atherosclerosis in the pathogenesis of dementia.⁵

The objective of the present study was to investigate the association of atherosclerosis and the risk of dementia prospectively, with non-invasive measures of atherosclerosis, including carotid intima media thickness (IMT), carotid plaques and peripheral arterial disease. The study was performed within the Rotterdam Study, a prospective population-based cohort study among persons aged 55 years or over.

Methods

Study population

The Rotterdam Study is a population-based prospective cohort study that investigates the incidence and risk factors of cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases in the elderly.⁶ From 1990-1993, all 10,275 residents aged 55 years or over of Ommoord, a district of the city of Rotterdam, were invited to participate in an extensive home interview and two visits to the research center, and 7,983 (78%) of them agreed. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. Follow-up examinations were conducted in 1993-1994, 1997-1999 and 2002-2004. In addition, through linkage with records of general practitioners, the total cohort was continuously monitored for morbidity and mortality. This resulted in a virtually complete follow-up until January 1, 2005. Assessment of atherosclerosis was performed during the baseline examination (1990-1993) and during the third survey (1997-1999). At baseline the study population consisted of 6647 persons who were cognitively screened, not demented and had assessment of at least 1 measure of atherosclerosis at baseline. Of this initial cohort at risk, 4139 persons visited the center during the third survey of whom 3962 were cognitively screened, not demented at that time and had assessment of at least 1 measure of atherosclerosis during the third survey.

Assessment of atherosclerosis

Carotid IMT and carotid plaques

Carotid plaques and vessel wall thickening was assessed with B-mode ultrasonography. Common carotid IMT was determined as the average of the maximum IMT of near-and far-wall measurements, and the average of left and right common carotid IMT was computed.⁷ Carotid plaques were determined at six different locations: common carotid artery, carotid bifurcation, and internal carotid artery at both left and right side.⁷ Data on carotid IMT and carotid plaques were available in 4521 and 5399 participants in the baseline examination. Data on carotid IMT and carotid plaques in the third survey were available in 3623 and 3339 persons.





Peripheral arterial disease

As indicator of peripheral arterial disease we measured the ankle-brachial index. The systolic blood pressure of the posterior tibial artery was measured on both sides with an 8-MHz continuous-wave Doppler probe (Huntleigh 500 D, Huntleigh Technology) and a random-zero sphygmomanometer. Sitting blood pressure of the right arm was measured twice with a random-zero sphygmomanometer. Mean blood pressure was used to calculate the ankle-arm index for each leg. In the analyses, we used the lowest measurement. Ankle-arm index was used as an indicator of peripheral arterial disease. Data on ankle-arm index were available in 6142 participants at the baseline examination and in 3775 participants at the third survey. Logistical reasons such as limited availability of ultrasonographers mainly accounted for the fact that not all measurements were available for all participants.

Diagnosis of dementia

During baseline and follow-up examinations the diagnosis of dementia followed a similar three-step protocol.^{8,9} Two brief tests of cognition (Mini-Mental State Examination (MMSE))¹⁰ and Geriatric Mental State schedule (GMS)¹¹ organic level were used to screen all participants. Screen-positives (MMSE score < 26 or GMS organic level > 0) underwent further cognitive testing using the Cambridge examination for mental disorders of the elderly (Camdex).¹² Persons who were suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for diagnosis. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.⁸ The diagnosis of dementia and subtype of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III-R),¹³ Alzheimer's disease (NINCDS-ADRD),¹⁴ and vascular dementia (NINDS-AIREN)¹⁵ by a panel of a neurologist, neuropsychologist and research physician.

Covariates

At baseline, trained investigators interviewed all participants at home, collecting information on current health status, medical history and smoking status. Additionally, at the research center, clinical measures were obtained. The body mass index (BMI) was calculated (weight (kg)/height (m²)). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. Non-fasting blood samples were drawn and immediately frozen. Total cholesterol, high-density lipid (HDL)-cholesterol and glucose were measured within 2 weeks. Quality of assessments was continuously monitored by Instruchemi® (Hilversum, the Netherlands). Genotyping for APOE was performed on coded DNA specimens without knowledge of diagnosis. Persons were





categorized on the basis of presence or absence of an APOE $\epsilon 4$ allele. We defined diabetes mellitus as a random or postload glucose level ≥ 11.1 mmol/l or the use of blood glucose lowering medication.

Data analysis

We examined the association of carotid IMT, carotid plaques and peripheral arterial disease, measured at the baseline examination, with risk of dementia and subtypes of dementia during follow-up by means of Cox' proportional hazard models. The association between carotid IMT and dementia was examined in quintiles using the first quintile as the reference category. Carotid plaques were analyzed per category, using no plaques as the reference category. Peripheral arterial disease was defined as ankle-brachial index lower than 0.90. Conform the previous report from the Rotterdam Study, a composite measure of atherosclerosis was constructed: a point was added to the atherosclerosis score if the following characteristics were present: carotid IMT in the highest quartile of the distribution, plaques in at least one of the common carotid arteries, evidence of peripheral arterial disease, defined as ankle-arm index less than 0.90. This atherosclerosis score was analyzed in four categories corresponding to score values of 0-3.⁴

In all analyses we adjusted for age and sex. To examine whether the associations may be explained by other cardiovascular factors, we additionally adjusted for systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol, and HDL-cholesterol. To evaluate competing risk of mortality we examined the associations between all indicators of atherosclerosis and mortality. In these analyses we created an outcome variable combining death and dementia, whichever came first. To examine whether the association between baseline atherosclerosis and risk of dementia differed across duration of follow-up, we performed separate analyses using incident cases identified until 1994 (second survey, 139 cases, mean (SD) follow-up 2.1 (0.8)), cases identified between 1995 and 2000 (third survey, 218 cases, mean (SD) follow-up 5.8 (1.6)) and those identified between 2000 and 2005 (fourth survey, 321 cases, mean (SD) follow-up 9.2 (3.3)). Next, we examined the associations between indicators of atherosclerosis measured at the third survey and risk of dementia thereafter.

Since an interaction between atherosclerosis and the APOE genotype on risk of dementia has been suggested,⁴ we examined whether the association between measures of atherosclerosis and dementia was different in carriers and non-carriers of the APOE $\epsilon 4$ allele. Because atherosclerosis is associated with stroke¹⁶ and stroke is associated with risk of dementia,¹⁷ we examined whether clinical stroke mediated the association by repeating the analyses excluding prevalent stroke and censoring incident stroke cases.



Results

During follow-up (mean (SD) 9.0 (3.4)) we identified 678 patients with incident dementia, of whom 476 patients were diagnosed with Alzheimer's disease, 52 patients with Alzheimer's disease and cerebrovascular disease and 78 patients with vascular dementia. Seventy-two patients developed dementia due to other causes. Table 1 shows the baseline characteristics of the study population. Compared with persons without an atherosclerosis measurement in the third survey, persons with at least one measurement of atherosclerosis were younger and healthier with respect to cardiovascular risk factors.

Table 1. Baseline characteristics (1990-1993) of the study population

	Total cohort	Persons eligible for third survey measurements*	
		Persons with measurement at third survey	Persons without measurement at third survey
N	6647	3962	1326
Age	69.0 (8.8)	65.7 (6.9)	70.2 (8.3)¶
Female sex %	59.3	58.5	69.1¶
Diabetes mellitus %	10.0	7.0	10.0¶
Body mass index kg/m ²	26.3 (4.0)	26.3 (4.0)	26.6 (3.9)
Diastolic blood pressure mmHg	73.7 (11.4)	73.6 (10.8)	74.6 (11.4)¶
Systolic blood pressure mmHg	139.3 (22.3)	135.7 (20.7)	142.5 (22.7)¶
Total cholesterol mmol/l	6.6 (1.2)	6.7 (1.2)	6.8 (1.2)
HDL-cholesterol mmol/l	1.3 (0.4)	1.4 (0.4)	1.4 (0.4)
Intima media thickness mm	0.79 (0.16)	0.76 (0.14)	0.79 (0.15)
Ankle brachial index	1.06 (0.23)	1.11 (0.18)	1.05 (0.22)¶
Current smoking %	22.9	21.8	24.2¶
Carotid plaques N (%)			
None	2217 (41.1)	1564 (47.3)	366 (38.1)¶
1-2	1822 (33.7)	1143 (33.7)	343 (35.7)
3-4	979 (18.1)	480 (14.5)	190 (19.8)
5-6	381 (7.1)	150 (3.7)	62 (6.5)

Values or means (standard deviation) or percentages

* Excluding persons who were demented or dead at time of invitation for third survey

¶ Significantly different ($p < 0.05$) from persons with atherosclerosis measurement at the third survey, after adjustment for age and sex (when applicable)



Table 2 shows that increased carotid IMT was associated with an increased risk of dementia. This increased risk was restricted to Alzheimer's disease. Other measures of atherosclerosis were not associated with risk of dementia and adjustment for cardiovascular factors did not markedly change associations. All indicators of atherosclerosis were strongly related to an increased risk of mortality. Figure 1 shows that increasing severity of atherosclerosis was associated with increased risk of dementia with short follow-up (until second survey). However, the p-trend was only borderline significant (0.08). No associations were found with risk of dementia in the third survey. Increasing atherosclerosis sumscores seemed associated with decreased risk of dementia with long follow-up (fourth survey), though the p-trend was not significant (0.66).

Table 3 shows that indicators of carotid atherosclerosis (carotid IMT and carotid plaques) measured at the third survey were strongly associated with an increased risk of dementia and Alzheimer's disease during follow-up until 2005. These analyses were based on 206 incident dementia cases (162 cases of Alzheimer's disease, 15 cases of Alzheimer's disease with cerebrovascular disease, 16 cases of vascular dementia and 13 cases of dementia due to other causes) that occurred during follow-up (mean (SD) 4.4 (1.0)). The associations remained after adjustment for cardiovascular factors. No significant associations were found with vascular dementia, probably due to a low number of cases.

No indication of interaction between APOE genotype and indicators of atherosclerosis was found (p-values for interaction terms between APOE genotype and carotid IMT, carotid plaques and peripheral arterial disease were 0.77, 0.37 and 0.11).

Exclusion of prevalent stroke cases and censoring incident stroke cases did not change the associations.

Discussion

In this population-based prospective cohort study, we found that atherosclerosis, predominantly carotid atherosclerosis, was associated with an increased risk of dementia. However, the associations were mainly confined to cases that occurred during shortterm follow-up. We found a strong association between all indicators of atherosclerosis and mortality.

The strengths of the Rotterdam Study include its prospective design, the population-based setting, its large number of participants, and its nearly complete follow-up. A potential limitation of the present study is that measures of atherosclerosis were not available in all participants. We showed that persons who had a measurement of atherosclerosis at the third survey were younger and had a better cardiovascular risk profile than persons who refused these measurements. Since these persons are less likely to die, competing risk of mortality may not have played a major role on the associations with dementias in these persons.



Table 2. The association between measures of atherosclerosis and risk of dementia during total follow-up. Hazard ratio (HR) (95% confidence interval (CI))¶ for dementia, subtypes of dementia and mortality or dementia whichever came first

	HR (95% CI) for dementia	HR (95% CI) for Alzheimer's disease	HR (95% CI) for vascular dementia	HR (95% CI) for mortality or dementia
Carotid IMT				
1 st quintile	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
2 nd quintile	1.20 (0.84-1.72)	1.22 (0.81-1.83)	1.12 (0.37-3.36)	1.14 (0.95-1.36)
3 rd quintile	1.15 (0.81-1.63)	1.20 (0.80-1.79)	1.17 (0.41-3.37)	1.18 (0.99-1.40)
4 th quintile	1.16 (0.81-1.64)	1.25 (0.84-1.86)	0.93 (0.32-2.73)	1.40 (1.18-1.65)
5 th quintile	1.50 (1.06-2.12)	1.54 (1.03-2.30)	1.33 (0.47-3.75)	1.54 (1.30-1.83)
Ptrend	0.03	0.04	0.65	<0.001
Carotid plaques				
None	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
1-2 plaques	1.04 (0.85-1.27)	1.03 (0.82-1.31)	0.99 (0.53-1.84)	1.16 (1.05-1.29)
3-4 plaques	1.05 (0.82-1.33)	1.02 (0.77-1.34)	1.31 (0.69-2.52)	1.37 (1.22-1.55)
5-6 plaques	0.98 (0.70-1.38)	1.11 (0.76-1.61)	0.96 (0.38-2.46)	1.84 (1.59-2.13)
Ptrend	0.90	0.68	0.66	<0.001
Peripheral arterial disease†				
	1.03 (0.85-1.25)	1.04 (0.84-1.29)	1.37 (0.81-2.34)	1.51 (1.38-1.65)
Atherosclerosis sumscore				
0	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
1	1.05 (0.83-1.34)	1.11 (0.84-1.46)	0.96 (0.47-1.94)	1.20 (1.07-1.36)
2	1.19 (0.88-1.61)	1.27 (0.90-1.79)	1.37 (0.63-3.01)	1.37 (1.18-1.59)
3	1.33 (0.87-2.03)	1.47 (0.91-2.37)	1.38 (0.45-4.19)	2.17 (1.80-2.63)
Ptrend	0.13	0.06	0.40	<0.001

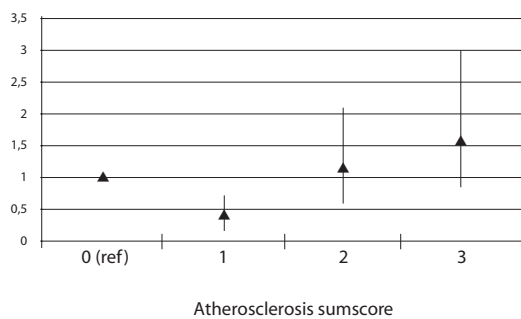
¶ Adjusted for age and sex

† Ankle-arm index <0.90

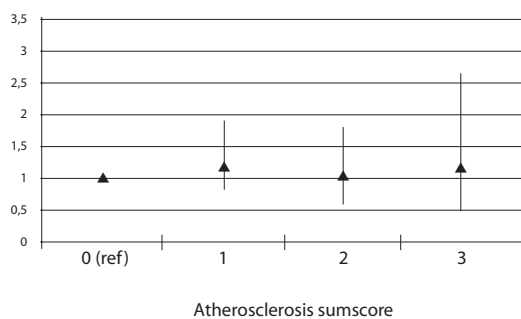


Figure 1. The association between baseline atherosclerosis severity, expressed as the atherosclerosis sumscore (0-3), and risk of incident dementia with onset between 1990-1995, 1995-2000 and 2000-2005. Hazard ratio (HR) and 95% confidence interval (CI) for dementia

HR (95% CI) for incident dementia with onset between 1990-1995



HR (95% CI) for incident dementia with onset between 1995-2000



HR (95% CI) for incident dementia with onset between 2000-2005

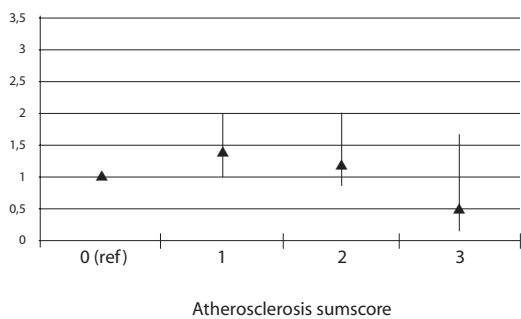


Table 3. Indicators of atherosclerosis measured at the third survey and risk of dementia. Hazard ratio and 95% confidence interval (HR (95% CI)¶)

	HR (95% CI) for dementia	HR (95% CI) for Alzheimer's disease	HR (95% CI) for vascular dementia
Carotid IMT			
1 st quintile	1.00 (reference)	1.00 (reference)	1.00 (reference)
2 nd quintile	2.25 (1.14-4.45)	2.06 (1.03-4.12)	-
3 rd quintile	2.94 (1.55-5.57)	2.34 (1.21-4.52)	-
4 th quintile	2.20 (1.14-4.25)	2.06 (1.06-4.01)	-
5 th quintile	2.60 (1.37-4.94)	2.40 (1.25-4.60)	-
Ptrend	0.03	0.03	
Carotid plaques			
None	1.00 (reference)	1.00 (reference)	1.00 (reference)
1-2 plaques	2.20 (1.35-3.61)	2.14 (1.27-3.61)	2.86 (0.34-24.49)
3-4 plaques	2.04 (1.21-3.44)	2.12 (1.22-3.69)	0.50 (0.03-8.29)
5-6 plaques	2.64 (1.34-5.22)	2.53 (1.21-5.32)	5.50 (0.54-56.06)
Ptrend	0.01	0.01	0.52
Peripheral arterial disease†			
	1.21 (0.89-1.68)	1.30 (0.92-1.84)	0.71 (0.20-2.56)
Atherosclerosis sumscore			
0	1.00 (reference)	1.00 (reference)	1.00 (reference)
1	1.18 (0.80-1.75)	1.30 (0.86-1.97)	0.68 (0.12-3.79)
2	1.32 (0.82-2.12)	1.35 (0.81-2.26)	1.60 (0.33-7.70)
3	1.74 (0.83-3.65)	1.91 (0.86-4.23)	2.09 (0.23-18.92)
Ptrend	0.09	0.07	0.44

¶ Adjusted for age and sex

† Ankle-arm index <0.90

It is difficult to differentiate between vascular dementia and Alzheimer's disease, and some misclassification may have occurred in classifying these subtypes.

Relatively few studies have examined the association of atherosclerosis with cognitive impairment and dementia¹⁸⁻²⁰ and limited evidence exists for a prospective association between atherosclerosis and risk of dementia.⁵ We previously reported on the cross-sectional



association between atherosclerosis and dementia.⁴ In other cross-sectional studies, carotid IMT was found to be weakly associated with poor cognitive performance in the general population¹⁹ and in stroke patients one year after stroke.²⁰ In a case-control study, Alzheimer's disease patients had significantly greater carotid IMT and a higher prevalence of multiple carotid plaques than did age-matched controls.¹⁸ Recently, in an autopsy series, a strong association was found between atherosclerotic cerebrovascular disease and increased frequency of neuritic plaques.²¹ In the prospective Cardiovascular Health Study, atherosclerosis, measured as IMT and ankle-arm index, was found to be a risk factor for Alzheimer's disease during follow-up of 5.4 years.⁵ Our findings of an association between atherosclerosis and risk of dementia are in line with these previous reports.

Our findings of an association predominantly with short follow-up only, in combination with evidence from cross-sectional studies may support the notion that the association between atherosclerosis and dementia is not causal. However, we found a strong association between atherosclerosis and mortality. Mortality related to atherosclerosis may explain the attenuated association during longer follow-up. This would imply that subjects with atherosclerosis who die during follow-up would have had an increased risk of dementia had they not died. In line with this view is our finding that the association between carotid IMT and dementia remained statistically significant during long follow-up. While other measures of atherosclerosis were more strongly related to mortality than to dementia, in the case of carotid IMT, the risk of mortality associated with carotid IMT was similar to the risk of dementia. This may have resulted in persistence of the association between carotid IMT and dementia during longer follow-up.

Our prospective study did not suggest interaction between APOE genotype and atherosclerosis. These findings are in contrast with our previous report⁴ but in line with the prospective study by Newman et al.⁵

Several mechanisms may explain an association between atherosclerosis and dementia. Cerebrovascular disease may mediate the association between atherosclerosis and dementia. Since excluding persons with a history of clinical stroke and censoring persons at the time of incident stroke did not affect the estimate, it is not likely that the association with dementia is solely explained by clinical stroke. Perhaps subclinical cerebrovascular disease, such as silent brain infarcts and periventricular white matter lesions mediate the association between atherosclerosis and dementia.^{2, 22-25} Because imaging of the brain was not routinely performed in all participants we were not able to assess this.

Also, atherosclerosis may induce cerebral hypoperfusion leading to cerebral hypoxia. These conditions may destabilize neurons and synapses and evolve in a neurodegenerative process characterized by formation of senile plaques, neurofibrillary tangles and amyloid angiopathy.^{1, 26} Another explanation for an association between atherosclerosis and dementia which has been suggested by Casserly et al. is that atherosclerosis and Alzheimer's disease





are independent processes with common elements in the causal pathway.²⁷ Although adjustment for cardiovascular risk factors in our study did not change the association between atherosclerosis and dementia, this explanation cannot be excluded. It is also possible that vascular events related to atherosclerosis accelerate the clinical expression of dementia in persons with existing Alzheimer's pathology.

An alternative explanation of our findings may be that specifically carotid IMT, but not other measures of atherosclerosis, is causally involved in the development of dementia. In this light it is important to note that carotid IMT and carotid plaques likely reflect different biological mechanisms. The main predictors of IMT are age and blood pressure while plaques are more strongly associated with lipids, smoking and other traditional risk factors.²⁸ It has been found that IMT strongly predicted stroke¹⁶ while carotid plaques were more closely related to myocardial infarction and coronary disease.²⁹ Though most predictors were controlled for and clinical stroke was taken into account, specifically carotid IMT may be associated with dementia through a different biological mechanism.

To conclude, our findings support the hypothesis that atherosclerosis is involved in the pathogenesis of dementia and Alzheimer's disease. Associations attenuated with increasing duration of follow-up, possible due to selective mortality in persons with severe levels of atherosclerosis.

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Chapter 3.2

Blood pressure and the risk of dementia. Results from the Rotterdam Study.

Abstract

Background Hypertension increases the risk of stroke and vascular dementia. For Alzheimer disease this relation is less clear. Studies with long follow up (10-25 years) suggest that hypertension increases the risk but studies with shorter follow-up have found the opposite, supposedly because blood pressure declines in the years preceding clinical onset of Alzheimer disease. We examined the association between blood pressure level and change in blood pressure level and the risk of dementia.

Methods The study is based on 6,140 participants aged 55 years and over of the population-based Rotterdam Study with blood pressure assessment at baseline (1990-1993) and who were still alive and non-demented at the first follow-up examination (1993-1994). The cohort was followed for incident dementia until January 1st 2005.

Results During 46,937 person-years of follow-up after the first follow-up examination 544 persons developed dementia. Overall, no association was found between baseline blood pressure level and dementia. However, for persons aged 55-75 years baseline blood pressure levels were associated with an increased risk of dementia (hazard ratio (HR) per standard deviation increase in SBP: 1.14, 95%CI 1.00-1.30; DBP: HR 1.16, 95%CI: 1.02-1.31). Persons whose blood pressure declined between baseline and first follow-up were at increased risk of dementia compared to persons with stable blood pressure levels (SBP: HR 1.44, 95%CI: 1.14-1.83; DBP: HR 1.42 95%CI: 1.09-1.84) regardless of age. Relations were strongest for vascular dementia, but also present for Alzheimer disease.

Conclusions These findings are compatible with the view that high blood pressure increases the risk of dementia and that a decline in blood pressure is related to risk of imminent clinical dementia.

Introduction

Hypertension is an established risk factor for stroke and vascular dementia.¹ Hypertension has also been suggested as a possible risk factor for Alzheimer disease.^{2,3} A link between hypertension and Alzheimer disease seems supported by a trial in which antihypertensive treatment in elderly people with isolated systolic hypertension was associated with a lower incidence of Alzheimer disease.⁴ Follow-up studies with long duration generally showed high blood pressure in midlife to be associated with an increased risk of Alzheimer disease.^{2,3,5,6}

By contrast, studies with short duration suggested that high blood pressure later in life is associated with a lower risk of Alzheimer disease.⁷⁻⁹ A possible explanation for these discrepant findings is that the blood pressure drops in the years preceding clinical onset of Alzheimer disease.





We examined the relation between blood pressure level and risk of dementia at intermediate duration of follow-up (median 6.3 years) and long follow-up (median 10.7 years). Furthermore, we evaluated the relation between decline in blood pressure over a 2-year period and the risk of dementia in the period thereafter (range 2.1-15.1 years). These associations were studied using data from the Rotterdam Study, a large population-based follow-up study conducted in the Netherlands that has been ongoing since 1990.

Methods

Study population

The Rotterdam Study is a population based prospective cohort study among 7,983 persons aged 55 years and older, including those living in institutions.¹⁰ The study was approved by the Medical Ethics Committee of the Erasmus Medical Center. Participants gave written informed consent and permission to retrieve information from treating physicians. At baseline (1990-1993),⁷ 528 subjects (94.3% of the total cohort) underwent extensive screening for dementia and 482 persons were identified with prevalent dementia. The cohort was re-examined in 1993-1994 (first follow-up) in 1997-1999 (second follow-up) and in 2002-2004 (third follow-up). In addition, the total cohort is continuously monitored for incident dementia cases via computerized linkage between the study database and medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care (RIAGG). This resulted in a virtually complete follow-up until January 1st, 2005. Of the 7,046 subjects at risk for dementia 6,668 had blood pressure measurements available at baseline. We excluded 10 persons of whom we were unable to obtain sufficient information on dementia status at the end of their second follow-up period. Since we hypothesized that the short-term relation between blood pressure and risk of dementia may be different from the relation over a longer period, we excluded the first follow-up period (14,127 person-years (median 1.9 years)) from the analyses. In this period 140 subjects developed dementia and an additional 378 died. Our analytical sample therefore effectively consisted of the 6,140 persons who were alive and non-demented at the first follow-up examination (Figure 1).

Diagnosis of dementia

The diagnosis of dementia was made following a three-step protocol.¹¹ Two brief tests of cognition (Mini-Mental State Examination (MMSE)¹² and Geriatric Mental State schedule (GMS)¹³ organic level) were used to screen all subjects. Screen-positives (MMSE score <26 or GMS organic level >0) underwent the Cambridge examination for mental disorders of the elderly (Camdex).¹⁴ Persons who were suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for diagnosis. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database





and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Healthcare. The diagnosis of dementia and subtype of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III)¹⁵ Alzheimer disease (NINCDS-ADRDA),¹⁶ and vascular dementia (NINDS-AIREN)¹⁷ by a panel of a neurologist, neuropsychologist and research physician.

Blood pressure and other baseline measurements

Baseline and follow-up blood pressure levels were measured in sitting position at the right upper arm with a random-zero sphygmomanometer. The average of two measurements, separated by a count of the pulse rate, was used in the analysis. At baseline and first follow-up participants were asked to report and show all medication used during the week preceding the interview. Subsequently, all drugs were classified according to their corresponding Anatomical-Therapeutic-Chemical-code (ATC-code).¹⁸

The following baseline variables were used as possible confounders: diabetes, defined according to WHO criteria for epidemiological studies of diabetes as the use of antidiabetes medication or a random or post-load serum glucose level greater than 11 mmol/l;¹⁹ education, dichotomized in primary education or less and more than primary education; smoking, categorized as never, past and current smoking; total cholesterol (mmol/l); body mass index (weight (kg)/height (m²)); and blood pressure lowering medication. A history of stroke was assessed at baseline and verified with medical records by a neurologist and a history of myocardial infarction was assessed by direct questioning and verified by ECG and general practitioner or cardiologist. Apolipoprotein E (APOE) genotyping was performed on coded DNA samples without knowledge of the diagnosis. The PCR product was digested with the restriction enzyme HhaI, and fragments were separated by electrophoresis.²⁰

Statistical analysis

The hazard ratios (HRs) of dementia by levels of baseline systolic and diastolic blood pressure were calculated with Cox' proportional hazards regression and presented with a 95% confidence interval (95% CI), controlling for age, age², and gender. This was done both by entering blood pressure as a categorical (quintiles) and as a continuous variable (per standard deviation) in the proportional hazards regression model. We performed separate analyses using incident dementia cases identified until 2000 (intermediate follow-up, median 6.3 years since baseline, range 2.1-10.0) and those identified between 2000 and 2004 (long follow-up, median 10.7 years since baseline, range 2.1-15.1). We examined if gender or age modified the relation in subanalyses where we stratified by gender and age (55-74, 75 years and older). In addition, age and gender were evaluated for potential interaction with blood pressure by adding separate terms to the regression models (age (continuously) x blood pressure (continuously); gender x blood pressure (continuously)). To check if associations could be





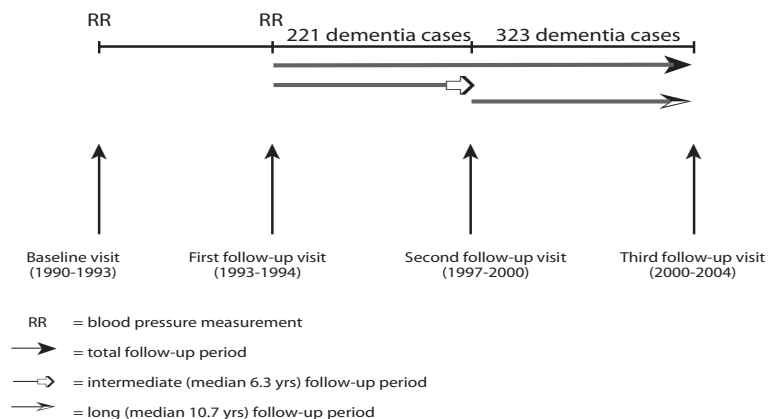
attributed to confounding, analyses were repeated with possible confounders added to the models. We repeated the analyses excluding persons on blood pressure lowering medication at baseline. To examine competing risk of mortality, we repeated the analyses with death or dementia as outcome variable. Next, to evaluate whether the associations may be explained by clinical stroke, we repeated the analyses excluding prevalent stroke cases and censoring incident stroke cases at time of stroke.

For the analysis on change in blood pressure level between baseline and first follow-up, we excluded 842 persons (13.7 %) who had no second blood pressure measurement. The risk of dementia was calculated using change in blood pressure per year as a categorical variable: increase (> 5 mm Hg increase), stable (increase ≤ 5 to a decrease < 5 mm Hg), decrease (decrease ≥ 5 mm Hg) in the proportional hazards regression model. All analyses were adjusted for age, age² and gender. Baseline blood pressure level may be correlated to change in blood pressure and hence be a confounder that should be adjusted for. On the other hand, adjusting for baseline level may cause bias due to regression towards the mean.²¹ We performed the analyses both with and without adjustment for baseline blood pressure level. Since the results did not differ from the unadjusted hazard ratios, we only present the analyses unadjusted for baseline blood pressure levels. All analyses were repeated adjusting for possible confounders. In addition, we repeated the analyses excluding persons on blood pressure lowering medication either at baseline or at first follow-up examination. Interactions terms of blood pressure lowering medication with SBP and DBP were computed.

Results

Baseline characteristics are given in Table 1. During a total of 46,937 person-years of follow-up 544 participants developed dementia. Alzheimer disease was diagnosed in 437 patients (401 without and 36 with cerebrovascular disease), vascular dementia was diagnosed in 55 patients and 52 subjects were diagnosed with other dementia. We identified 221 incident

Figure 1



dementia cases before January 1st 2000 and 323 cases between January 1st 2000 and January 1st 2005.

Non-responders to the first follow-up examination were on average older, were more frequently female and a larger percentage developed dementia at follow-up. Of a total of 221 incident dementia cases, 55 (24.9%) did not respond to the first follow-up, whereas 722 (12.2%) of 5919 non-demented did not respond to the first follow-up. Non-responders had higher baseline systolic and diastolic blood pressure levels compared to responders. Mean change in systolic blood pressure was 1.1 mm Hg increase per year (SD 10.8), mean change in diastolic blood pressure was 1.4 mm Hg increase per year (SD 6.5).

Analyses in quintiles of systolic and diastolic blood pressure showed a linear relation between baseline blood pressure and risk of dementia. Therefore we entered blood pressure as a continuous variable (per standard deviation) in the models. Adjusted hazard ratios of

Table 1. Baseline characteristics (1990-1993) of participants of the Rotterdam Study who were alive and non-demented at first follow-up (1993-1994) and who had blood pressure assessed at baseline.

Baseline characteristics	Total Population (n = 6,140)	55-74 years (n = 4,817)	≥ 75 years (n = 1,323)
Age (years)	68.2 (8.3)	64.8 (5.4)	80.4 (4.3)
Gender (% women)	59.6	57.3	68.0
Blood pressure lowering drugs (%)	30.3	26.7	43.4
Systolic blood pressure (mm Hg)	139.1 (22.1)	136.7 (21.4)	147.8 (22.6)
Diastolic blood pressure (mm Hg)	73.9 (11.3)	74.3 (11.1)	72.7 (12.0)
Diabetes (%)	9.4	8.0	14.5
Smoking : Current (%)	22.8	25.2	14.0
Former (%)	42.5	44.4	35.6
Never (%)	34.6	30.4	50.4
Primary education or less (%)	35.9	30.9	54.4
Total cholesterol (mmol/l)	6.7 (1.2)	6.7 (1.2)	6.5 (1.3)
Body mass index (kg/m ²)	26.4 (4.0)	26.3 (4.0)	26.6 (4.0)

Figures are proportions (%) or means (SD)

dementia and subtypes of dementia per standard deviation increase in baseline blood pressure level are given in Table 2a for systolic blood pressure and Table 2b for diastolic blood pressure. Overall increasing baseline systolic and diastolic blood pressure levels were not associated with dementia or Alzheimer disease. The risk of vascular dementia increased with increasing

Table 2a. Adjusted hazard ratio for subtypes of dementia with onset between 2.1 and 9.8 years after baseline associated with baseline blood pressure level*

Systolic BP (per SD)	Overall (2.1-9.8 years)	Intermediate (2.1-6.2 years)	Long (6.2-9.8 years)
	follow-up	follow-up	follow-up
Total population	All dementia 1.02 (0.93-1.11)	1.12 (0.97-1.28)	0.95 (0.84-1.08)
	Alzheimer's disease 0.97 (0.87-1.08)	1.06 (0.90-1.25)	0.90 (0.79-1.04)
	Vascular dementia 1.22 (0.93-1.60)	1.47 (1.05-2.07)	0.93 (0.59-1.46)
55-74 years	All dementia 1.14 (1.00-1.30)	1.47 (1.17-1.84)	1.00 (0.85-1.18)
	Alzheimer's disease 1.10 (0.94-1.28)	1.39 (1.06-1.82)	0.98 (0.81-1.19)
	Vascular dementia 1.61 (1.05-2.47)	1.85 (1.04-3.28)	1.21 (0.60-2.45)
≥ 75 years	All dementia 0.92 (0.81-1.05)	0.93 (0.77-1.12)	1.00 (0.85-1.19)
	Alzheimer's disease 0.89 (0.77-1.03)	0.92 (0.74-1.13)	0.95 (0.79-1.15)
	Vascular dementia 0.99 (0.69-1.41)	1.12 (0.71-1.77)	0.66 (0.34-1.28)

* Adjusted for age, gender, smoking, diabetes, cholesterol, body-mass-index, education, blood pressure lowering medication, history of stroke, history of myocardial infarction, APOE genotype

Table 2b. Adjusted hazard ratio for subtypes of dementia with onset between 2.1 and 9.8 years after baseline associated with baseline blood pressure level*

Diastolic BP (per SD)	Overall (2.1-9.8 years)	Intermediate (2.1-6.2 years)	Long (6.2-9.8 years)
	follow-up	follow-up	follow-up
Total population	All dementia 1.06 (0.97-1.16)	1.10 (0.96-1.25)	1.05 (0.94-1.18)
	Alzheimer's disease 1.01 (0.91-1.11)	1.04 (0.89-1.21)	0.99 (0.86-1.13)
	Vascular dementia 1.30 (1.00-1.69)	1.42 (1.02-1.98)	1.15 (0.75-1.77)
55-74 years	All dementia 1.16 (1.02-1.31)	1.32 (1.06-1.65)	1.10 (0.94-1.28)
	Alzheimer's disease 1.08 (0.92-1.26)	1.18 (0.90-1.35)	1.04 (0.86-1.26)
	Vascular dementia 1.90 (1.28-2.82)	2.35 (1.38-4.02)	1.34 (0.70-2.56)
≥ 75 years	All dementia 1.00 (0.88-1.13)	1.00 (0.84-1.19)	1.00 (0.85-1.19)
	Alzheimer's disease 0.98 (0.85-1.12)	0.99 (0.81-1.21)	0.95 (0.79-1.15)
	Vascular dementia 0.97 (0.67-1.40)	0.95 (0.59-1.51)	0.78 (0.41-1.48)

* Adjusted for age, gender, smoking, diabetes, cholesterol, body-mass-index, education, blood pressure lowering medication, history of stroke, history of myocardial infarction, APOE genotype

blood pressure levels. The strongest relation was found for the short follow-up and stronger for diastolic than for systolic blood pressure. Because of a significant interaction between age and systolic blood pressure ($p = 0.127$ for overall and $p = 0.010$ for intermediate follow-up) we examined the association between blood pressure and dementia stratified for age above and below 75 years of age. In younger subjects, increasing baseline systolic and diastolic blood pressure levels were associated with an increased risk of dementia, whereas in older subjects no association was found. When we stratified on duration of follow-up, the risk was restricted to intermediate follow-up. We found that high systolic blood pressure was associated with an increased risk of death or dementia (HR per standard deviation increase in SBP: 1.11, 95% CI: 1.07-1.16 for the total population, 1.13, 95% CI 1.00-1.20 in persons younger than 75 years and 1.10, 95% CI 1.03-1.17 in persons of 75 years and over). High diastolic blood pressure was also associated with an increased risk of death or dementia (HR per standard deviation increase in DBP: 1.08, 95% CI: 1.04-1.13 for the total population, 1.08, 95% CI 1.02-1.14 in persons younger than 75 years and 1.08, 95% CI 1.02-1.15 in persons of 75 years and over). The association between blood pressure and dementia was largely attributed to vascular dementia, although we also observed an association with systolic blood pressure and Alzheimer disease on intermediate follow-up. Gender specific analyses revealed no differences regarding the risk of Alzheimer disease or vascular dementia. After exclusion of persons who used blood pressure lowering medication at baseline the hazard ratios did not change appreciably (interaction between SBP or DBP and blood pressure lowering medication: $p = 0.21$, $p = 0.92$). Associations did not change markedly after excluding prevalent stroke and censoring incident stroke cases. In particular, the HR for Alzheimer disease per standard deviation increase in SBP in persons younger than 75 years with intermediate duration of follow-up was similar (1.39, 95% CI: 1.03-1.87).

Tables 3a (for systolic) and 3b (for diastolic) show the relative risk of dementia associated with change in blood pressure level from baseline to first follow-up in categories of change in blood pressure level. A decline in systolic or diastolic blood pressure level was associated with an increased risk of dementia at follow-up. This relation was present for both Alzheimer disease (decline in SBP: HR 1.31, 95% CI: 0.99-1.73; decline in DBP: 1.39, 95% CI: 1.02-1.89) and vascular dementia (decline in SBP: HR 2.62, 95% CI: 1.17-5.83; decline in DBP: 1.94, 95% CI: 0.90-4.16). The strongest associations were found for the short follow-up. The association was not significantly different for men and women and similar for younger and older persons. The analysis in which we excluded persons who used blood pressure lowering medication at baseline or follow-up yielded practically similar results for decline in systolic blood pressure (interaction of blood pressure lowering medication with SBP: $p=0.51$, with DBP: $p=0.24$).

Discussion

We found that persons with high blood pressure levels at baseline were at increased risk of dementia. These findings were restricted to those younger than 75 years. The association could largely be attributed to the increased risk of vascular dementia, but we also observed a significant relation between systolic blood pressure and Alzheimer disease. The lack of association with dementia in the long follow-up might have been due to selective mortality among those with high blood pressure levels. Those with a decline in blood pressure were at increased risk of dementia in the years immediately thereafter, independent of age, gender or blood pressure lowering medication.

The strength of our study is that we followed a large cohort of elderly subjects with repeated detailed examinations including dementia screening over a period of maximal fifteen years and that follow-up was virtually complete. This allowed us to investigate change in blood pressure level in relation to risk of dementia. However, there are some methodological issues that need to be discussed. First, subjects had to survive long enough to obtain a second blood pressure measurement. Participants who died between baseline examination and first follow-up may have had a more severe decline in blood pressure compared with those

Table 3a. Adjusted hazard ratio for subtypes of dementia (Alzheimer's disease and vascular dementia) associated with a change in systolic blood pressure from baseline to follow-up (n = number of cases)*

		Overall (2.1-9.8 years follow-up)	Short (2.1-6.2 years follow-up)	Long (6.2-9.8 years follow-up)
All dementia	(cases)	(446)	(165)	(281)
	Increase	1.10 (0.88-1.37)	1.22 (0.83-1.79)	1.05 (0.80-1.38)
	Stable	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Decrease	1.44 (1.14-1.83)	2.20 (1.54-3.14)	1.21 (0.89-1.64)
Alzheimer's disease	(cases)	(341)	(125)	(216)
	Increase	1.11 (0.86-1.42)	1.11 (0.72-1.71)	1.11 (0.82-1.51)
	Stable	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Decrease	1.31 (0.99-1.73)	1.59 (1.01-2.50)	1.13 (0.79-1.61)
Vascular dementia	(cases)	(41)	(22)	(19)
	Increase	1.55 (0.69-3.48)	2.02 (0.57-7.16)	1.15 (0.39-3.33)
	Stable	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Decrease	2.62 (1.17-5.83)	4.65 (1.40-15.50)	1.22 (0.37-3.98)

* Adjusted for age, age², gender, smoking, diabetes, cholesterol, body-mass-index, education, blood pressure lowering medication, history of stroke, history of myocardial infarction, APOE genotype

† Increase: increase > 5 mm Hg; stable: increase ≤ and decrease < 5 mm Hg; decrease: ≥ 5 mm Hg

Table 3b. Adjusted hazard ratio for subtypes of dementia (Alzheimer's disease and vascular dementia) associated with a change in diastolic blood pressure from baseline to follow-up (n = number of cases)*

		Overall (2.1-9.8 years follow-up)	Short (2.1-6.2 years follow-up)	Long (6.2-9.8 years follow-up)
All dementia	(cases)	(446)	(165)	(281)
	Increase	0.87 (0.69-1.09)	0.80 (0.54-1.20)	0.89 (0.67-1.19)
	Stable	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Decrease	1.42 (1.09-1.84)	1.72 (1.16-2.57)	1.20 (0.85-1.70)
Alzheimer's disease	(cases)	(341)	(125)	(216)
	Increase	0.90 (0.69-1.17)	0.93 (0.60-1.43)	0.86 (0.62-1.20)
	Stable	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Decrease	1.39 (1.02-1.89)	1.58 (0.98-2.54)	1.21 (0.81-1.82)
Vascular dementia	(cases)	(41)	(22)	(19)
	Increase	0.75 (0.33-1.71)	0.37 (0.08-1.65)	1.32 (0.45-3.82)
	Stable	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Decrease	1.94 (0.90-4.16)	1.10 (0.71-5.22)	1.91 (0.56-6.46)

* Adjusted for age, age², gender, smoking, diabetes, cholesterol, body-mass-index, education, blood pressure lowering medication, history of stroke, history of myocardial infarction, APOE genotype

† Increase: increase > 5 mm Hg; stable: increase ≤ and decrease < 5 mm Hg; decrease: ≥ 5 mm Hg

who survived.²² This could, however, only have led to an overestimation of the relative risk of dementia if the mortality rate was higher in non-demented than in demented, which we consider very unlikely. Second, persons who did not attend the first follow-up more often developed dementia in the period thereafter. However, since persons who developed dementia at follow-up were on average much older and high age is strongly correlated to lower response rates, we believe that this difference in response rates was mainly due to age.

Our findings lend further support to the notion that high blood pressure may increase the long-term risk of low cognitive performance²³⁻²⁶ and dementia.^{2, 3, 6} Several possible explanations for the long-term increased risk of dementia in participants with high baseline blood pressure levels may be offered. First, hypertension is a major risk factor for stroke and single strategic or multiple infarcts may lead to vascular dementia.²⁷ Second, hypertension is the dominant risk factor for cerebral white matter lesions.²⁸⁻³⁰ These lesions are associated with cognitive dysfunction in non-demented subjects,³¹ and are abundantly present in demented subjects.^{32, 33} Third, hypertension can lead to endothelial damage and impaired blood brain barrier function.³⁴ The latter may impair the transport of crucial nutrients and metabolites to



the brain or allow circulating toxic agents to gain access to the brain tissue. It still remains to be elucidated whether and how this may eventually contribute to the development of Alzheimer disease.^{33, 34} The association between high blood pressure and Alzheimer disease did not change when we took clinical stroke into account. Perhaps, a proportion of dementia cases may be a combination of Alzheimer pathology and lesions of vascular origin (silent cerebral infarction).³⁵

We observed a relation between increasing blood pressure and Alzheimer disease only during intermediate follow-up. In the period between on average 6.2 and 9.8 years no relation was present, probably due to selective mortality in those with high blood pressure. These findings are in contrast with several studies with longterm follow-up showing an association between high blood pressure in midlife and increased risk of Alzheimer disease in late-life.^{2, 3, 5, 6} Those with a decline in blood pressure had a short-term increased risk of dementia, irrespective of baseline blood pressure level. A possible explanation is that blood pressure declines as a consequence of brain lesions associated with developing dementia, since several areas that are involved in central blood pressure regulation are affected in Alzheimer disease.³⁶⁻³⁸ A second, but equally possible, explanation is that lowering of the blood pressure beyond a critical threshold increases the risk of dementia through chronic hypoperfusion, especially in elderly persons with longstanding hypertension.³⁹ This explanation would fit with the observation that relative hypoperfusion is associated with risk of imminent dementia.⁴⁰ Our study suggests that it is important to closely monitor blood pressure levels in the elderly and supports the hypothesis that early control of high blood pressure may reduce the risk of dementia in the elderly.⁴¹

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Chapter 3.3

Arterial Stiffness, cognitive decline and risk of dementia. The Rotterdam Study

Abstract

Background and Purpose Arterial stiffness is associated with an increased risk of myocardial infarction and stroke, independent of classical vascular risk factors. Vascular factors and stroke are associated with cognitive function and dementia. We examined whether arterial stiffness was independently associated with cognitive function and dementia.

Methods The present study was based on the Rotterdam Study, a prospective population-based cohort study ongoing since 1990. During the third examination (1997-1999) arterial stiffness was measured by assessment of pulse wave velocity and carotid distensibility. Cognitive function was assessed during the third and fourth examination (2002-2004) with a neuropsychological test battery. We used linear and logistic regression to estimate the association of arterial stiffness with cognitive function and cognitive decline. From the third examination until January 1st 2005 we identified 156 incident dementia cases. Cox' proportional hazard models were used to estimate the association between arterial stiffness and the risk of dementia.

Results After adjustment for cardiovascular risk factors we found an association of increased pulse wave velocity with poorer performance on the Stroop test (adjusted β -coefficient (95% confidence interval) 1.13 (0.25-2.00) per standard deviation increase in pulse wave velocity) but not with performance on other cognitive tests. No associations were found between measures of arterial stiffness and cognitive decline or risk of dementia after adjustment for cardiovascular factors.

Conclusions We failed to identify arterial stiffness as an independent risk factor of cognitive decline or risk of dementia.

Introduction

Arterial stiffness is a predictor of cardiovascular disease and mortality. Recently, the aortic pulse wave velocity index, a measure of arterial stiffness, was found to be of added value above traditional cardiovascular risk factors in the prediction of coronary heart disease and stroke.¹ Elevated arterial stiffness is a result of structural and functional changes of the vessel wall that occur with aging. Furthermore, higher arterial stiffness is associated with higher systolic blood pressure, increased pulse pressure and atherosclerosis.²

Many studies have demonstrated an association of vascular factors and cerebrovascular disease with dementia and cognitive decline.³ Recently, some studies reported an association between increased arterial stiffness, measured by pulse wave velocity (PWV), and poor cognitive function and suggested that arterial stiffness may be a determinant of cognitive decline and dementia.⁴⁻⁶ However, these studies were all small, cross-sectional, and mostly performed





in selected clinic-based samples. To date, no prospective studies have been reported that examined the association of arterial stiffness with cognitive decline or dementia.

In this study we set out to investigate the association between arterial stiffness and cognitive function, cognitive decline and risk of dementia in the general population. Furthermore, to investigate whether arterial stiffness may be an independent risk factor of cognitive decline and dementia, we examined whether these associations were independent of cardiovascular factors.

Methods

Study sample

This study was based on the Rotterdam Study, a prospective population-based cohort study among 7,983 elderly aged 55 years and older.⁷ Baseline examinations were performed from 1990 through 1993. Participants were interviewed at their homes and subsequently examined at the research center. Follow-up examinations were conducted in 1993-1994, 1997-1999 and in 2002-2004. The Medical Ethics Committee of Erasmus Medical Center approved the study, and written informed consent was obtained from all participants. Arterial stiffness was first measured during the third survey in 1997-1999 in 3779 persons of the 4024 persons who visited the research center. Missing information on PWV or carotid distensibility was almost entirely due to logistic reasons, in particular malfunctioning equipment or unavailability of technicians. Cognitive function was assessed at the third examination in 1997-1999 and the fourth examination in 2002-2004.

We excluded individuals with dementia at the time of the third examination, which left 3714 persons who had arterial stiffness measurements, underwent neuropsychological testing and were not demented at the third examination to be included in our analyses. Follow-up for incident dementia was virtually complete until January 1st 2005. Of the 3714 persons, 947 persons did not visit the research center for the fourth examination. Of these, 527 persons had died and 420 persons refused to visit the center. As a result, the analyses regarding arterial stiffness and change in cognitive function were based on 2767 persons who underwent neuropsychological testing during 1997-1999 and 2002-2004.

Measures of arterial stiffness

Pulse wave velocity

Carotid-femoral PWV, a measure of aortic stiffness, was measured with persons in supine position. PWV was assessed with an automatic device (Complior[®] Artech Medica, Pantin, France).⁸ The time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid artery and the femoral artery was recorded. The distance between recording sites in the carotid and the femoral arteries (the carotid artery and the groin) was measured with a tape over the surface of the body. The ratio between the foot-to-foot delay





and the distance covered by the pulse wave is the pulse wave velocity and is expressed in meters per second.

Carotid distensibility

Carotid distensibility was measured at the right common carotid artery with the subjects in supine position and the head slightly tilted to the contra lateral side. The vessel wall motion was assessed with a duplex scanner (ATL Ultra mark IV, operation frequency 7.5 MHz) connected to a vessel wall movement detector system.⁹ After 5 minutes of rest, a region at 1.5 cm proximal to the origin of the bulb of the artery was identified using B-mode ultrasound. The displacement of the arterial walls was obtained by processing the radio frequency signals originating from two selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter ($\Delta D/D$), were computed as the mean of four cardiac cycles of three successive recordings. Blood pressure was measured twice with a Dinamap automatic blood pressure recorder during the measurement session. The mean was taken as the person's reading. Pulse pressure (ΔP) was defined as the difference between systolic and diastolic blood pressure. The cross-sectional arterial wall distensibility coefficient was calculated according to the following equation: distensibility coefficient = $2 (\Delta D/D) / \Delta P$ (10^{-3} kPa). In a reproducibility study performed among 47 subjects, the intraclass correlation coefficient was 0.80 for both the PWV and the carotid distensibility coefficient.

Assessment of cognitive function

The Mini Mental State Examination (MMSE) is a widely used test for global cognitive function.¹⁰ Executive cognitive function was measured with the Letter-Digit Substitution Task (LDST), an abbreviated Stroop test and the Word Fluency Test (WFT). The LDST is a modified version of the Symbol Digit Modalities Test¹¹ and asks the participants to make as many letter-digit combinations as possible in 60 seconds. The abbreviated Stroop test consists of three subtasks in which the participant is shown a card with 40 items that have to be named.¹² The first card contains color-names, printed in black; the second card contains colored blocks; the third card contains color-names, printed in a different color than the color-name. As an outcome we used time needed for the third trial in which the participants are asked to name the color in which the color-name is printed. In the WFT, used to test verbal fluency, participants were asked to name as many animals as possible within 60 seconds.

Diagnosis of dementia

The diagnosis of dementia was made following a three-step protocol.¹³ Two brief tests of cognition (MMSE and Geriatric Mental State schedule (GMS) organic level) were used to screen all subjects. Screen-positives (MMSE score < 26 or GMS organic level > 0) underwent





the Cambridge examination for mental disorders of the elderly (Camdex). Subjects who were suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for diagnosis. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. The diagnosis of dementia and subtypes of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III-R), Alzheimer's disease (AD) (NINCDS-ADRDA), and vascular dementia (NINDS-AIREN) by a panel of a neurologist, a neuropsychologist and a research physician.

Covariates

Level of education was obtained during the baseline interview and dichotomized into primary education or less and more than primary education. At the research center clinical measures were obtained. Systolic and diastolic blood pressures were measured twice on the right arm with a random-zero sphygmomanometer, after the participant had been seated for at least 5 minutes. The mean of the 2 blood pressure values was used in the analyses. Pulse pressure was defined as the difference between systolic and diastolic blood pressure. Mean arterial pressure was calculated as diastolic blood pressure plus one-third of the pulse pressure. The body mass index (BMI) was calculated (weight (kg)/length (m²)). Fasting serum total and high-density lipoprotein (HDL) cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System). Genotyping for APOE was performed on coded DNA specimens without knowledge of diagnosis. Persons were categorized on the basis of presence or absence of an APOE $\epsilon 4$ allele. Persons with the APOE $\epsilon 2/\epsilon 4$ genotype were excluded from the analyses. Furthermore, ultrasonography of both carotid arteries was performed. As an indicator of atherosclerosis of the carotid arteries we used intima-media thickness. Common carotid intima-media thickness (IMT) was determined as the average of the maximum IMT of near-and far-wall measurements, and the average of left and right common carotid IMT was computed. Diabetes mellitus was defined as the use of blood glucose-lowering medication and/or fasting serum glucose level ≥ 7.0 mmol/l.

Data analysis

First, we examined the association of PWV and carotid distensibility per standard deviation (SD) increase with cognitive function by means of linear regression models, adjusted for age, sex and education. Further adjustment was made for mean arterial pressure and heart rate as these measures have a direct effect on arterial stiffness. Then, to examine whether associations were independent of other vascular factors, we adjusted for BMI, smoking, IMT, total cholesterol, HDL cholesterol and diabetes mellitus.





Next, we examined the association between arterial stiffness and cognitive decline with logistic regression models. Decline on cognitive tests was defined as a negative difference between the test scores from the third and fourth examination larger than 1 SD of the mean difference. Analyses were adjusted as described for the cross-sectional analyses. Since stroke has been related to cognition and an association has been reported between arterial stiffness and risk of stroke,¹ we adjusted for prevalent and incident strokes in additional analyses. To assess whether associations differed across age categories we repeated the analyses in strata of age (≤ 75 years and > 75 years). We repeated the analyses adjusting for cognitive function at baseline. Also, analyses were repeated excluding 89 persons, of the 2767 persons included in the population for analyses regarding change in cognitive function, who had become demented during follow-up.

Finally, we used Cox' proportional hazard models to examine the association between arterial stiffness and the risk of dementia and subtypes of dementia. Follow-up time was defined as the time of arterial stiffness measurement until dementia diagnosis, death or end of study, whichever came first. We examined the association of PWV and carotid distensibility with dementia per SD increase of PWV and carotid distensibility. We adjusted for age, sex and education, and subsequently for mean arterial pressure, heart rate and cardiovascular risk factors. To investigate whether APOE genotype modified the association of PWV and carotid distensibility with dementia, we examined the association in strata of carriers and non-carriers of the APOE $\epsilon 4$ allele and computed interaction terms between measures of arterial stiffness and the APOE genotype.

All analyses were performed using the statistical package SPSS 11.0 for Windows (SPSS Inc, Chicago, Illinois, USA).

Results

Characteristics of persons who visited the center during the third and fourth examination are shown in table 1. Persons who participated in both the third and the fourth examination had a better cardiovascular risk profile and performed better on all cognitive tests compared to persons who only participated in the third examination also when differences in age were taken into account. PWV and carotid distensibility were normally distributed and inversely correlated (Spearman's correlation coefficient -0.41 , p -value < 0.001).

After adjustment for age, sex and education, statistically significant associations were found for increased PWV and worse performance on the MMSE, the Stroop test and the WFT and for decreased carotid distensibility and worse performance on the MMSE and the Stroop test (model 1 in table 2).

Adjustments for mean arterial pressure and heart rate attenuated all associations. The associations were attenuated further after adjusting for cardiovascular factors and only the association between PWV and worse performance on the Stroop test remained statistically significant (model 2 in table 2).





Table 1. Baseline characteristics of the study population, persons who participated in the third examination (n=3714), persons who participated in the third examination but not in the fourth examination (n=947) and persons who participated in both examinations (n=2767)

	Third examination n=3714	Third but not fourth examination n=947	Third and fourth examination n=2767
Men (%)	42.3	43.6	41.9
Age (years) (SD*)	72.0 (6.7)	75.7 (7.3)	70.7 (6.0)
Primary education (%)	29.0	34.9	27.0
Prevalent stroke cases (%)	3.8	7.0	2.7
Pulse Wave Velocity (m/s) (SD)	13.5 (3.0)	14.5 (3.3)	13.2 (2.9)‡
Distensibility Coefficient (10 ⁻³ kPa) (SD)	10.5 (4.4)	9.2 (4.2)	10.9 (4.3)
Mean Arterial Pressure (mmHg) (SD)	106.7 (12.8)	108.2 (13.7)	106.1 (12.5)‡
Heart Rate (bpm) (SD)	75.2 (14.6)	77.2 (15.4)	74.5 (14.2)‡
Diabetes Mellitus (%)	10.9	16.3	9.0‡
Body Mass Index (kg/m ²) (SD)	26.8 (4.0)	26.7 (4.1)	26.9 (3.9)
Current Smokers (%)	16.0	18.8	15.1‡
Total Cholesterol (mmol/L) (SD)	5.8 (1.0)	5.8 (1.0)	5.9 (1.0)
HDL-Cholesterol (mmol/L) (SD)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)‡
Intima Media Thickness (mm) (SD)	0.9 (0.2)	0.9 (0.2)	0.9 (0.1)‡
Mini Mental State Examination (SD)	27.7 (2.0)	26.9 (2.6)	28.0 (1.6)‡
Word Fluency Test (number of correct answers) (SD)	20.9 (5.5)	19.0 (5.5)	21.5 (5.3)‡
Stroop Test (seconds) (SD)	57.2 (21.1)	66.0 (27.2)	54.4 (17.8)‡
Letter Digit Substitution Test (number of correct answers) (SD)	26.6 (7.1)	23.4 (7.5)	27.7 (6.6)‡

* SD= standard deviation ‡Age and sex adjusted difference between the groups <0.05



Table 2. Association of arterial stiffness as measured through pulse wave velocity (PWV) and carotid distensibility (CD) with cognitive function, using linear regression models

	Model 1				Model 2			
	Difference in test scores (95% CI)				Difference in test scores (95% CI)			
	MMSE	Letter-Digit Substitution Test	Stroop Test	Word Fluency Test	MMSE	Letter-Digit Substitution Test	Stroop Test	Word Fluency Test
PWV per SD	-0.08 (-0.15;-0.01)	-0.19 (-0.42;0.05)	1.39 (0.65;2.13)	-0.31 (-0.50;-0.12)	-0.05 (-0.13;0.03)	-0.08 (-0.36;0.19)	1.13 (0.26;1.99)	-0.18 (-0.41;0.05)
CD per SD	0.09 (0.01;0.16)	0.12 (-0.13;0.36)	-0.82 (-1.56;-0.08)	0.14 (-0.06;0.35)	0.04 (-0.06;0.13)	-0.04 (-0.34;0.26)	-0.30 (-1.20;0.61)	-0.08 (-0.34;0.17)

Model 1: adjusted for age, sex and education

Model 2: additionally adjusted for mean arterial pressure, heart rate, current smoking, diabetes mellitus, body mass index, total cholesterol, high density lipid cholesterol and intima media thickness

Table 3. Association between arterial stiffness (pulse wave velocity (PWV) and carotid distensibility (CD)) and cognitive decline (odds ratio (OR) and 95 % confidence interval (CI) per standard deviation (SD) increase), using logistic regression models

		Model 1			Model 2		
		OR for decline (95% CI)			OR for decline (95% CI)		
		MMSE	Letter-Digit Substitution Test	Stroop Test	Word Fluency Test	MMSE	Letter-Digit Substitution Test
PWV per SD		0.98 (0.86-1.12)	1.14 (1.00-1.31)	1.00 (0.85-1.17)	1.07 (0.95-1.21)	0.93 (0.79-1.09)	1.09 (0.92-1.28)
CD per SD		0.94 (0.81-1.09)	0.90 (0.78-1.04)	0.99 (0.83-1.18)	0.91 (0.80-1.03)	0.99 (0.82-1.19)	0.89 (0.75-1.07)
						0.89 (0.73-1.08)	1.10 (0.89-1.37)
							1.07 (0.93-1.23)
							0.97 (0.84-1.13)

Model 1: adjusted for age, sex and education
Model 2: additionally adjusted for mean arterial pressure, heart rate, current smoking, diabetes mellitus, body mass index, total cholesterol, high density lipid cholesterol and intima media thickness

No association was found between arterial stiffness and cognitive decline (models 1 and 2 in table 3). The incidence rate of stroke in the Rotterdam Study was 9.4 per 1000 personyears.¹⁴ Additional adjustment for prevalent and incident stroke did not change the associations. The associations were similar for younger and older people (≤ 75 years, > 75 years). Associations did not change after adjusting for baseline cognitive function and after exclusion of persons who had become demented.

The incidence rate of dementia in the Rotterdam Study was 9.8 per 1000 personyears.¹⁵ During a mean (SD) follow-up of 4.4 (0.9) years we identified 156 persons with incident dementia (including 89 persons who visited the research center during the fourth survey and 67 patients who were identified through medical records), of whom 136 persons were diagnosed with Alzheimer's disease and 11 persons with vascular dementia.

Table 4 shows that PWV and carotid distensibility were not associated with risk of dementia. For AD the hazard ratio (95% CI) was 0.90 (0.75-1.07) per SD increase in PWV. For vascular dementia there seemed to be an association (hazard ratio (95% CI) 1.47 (0.94-2.30)) per SD increase in PWV, though this was not significant, possibly due to the low number of vascular dementia cases. Per SD increase in carotid distensibility the hazard ratio (95% CI) for AD was 1.14 (0.91-1.43) and 0.58 (0.21-1.57) for vascular dementia. The associations did not differ between carriers and non-carriers of the APOE $\epsilon 4$ allele (p-value of the interaction term between PWV and APOE genotype was 0.89 and 0.94 between carotid distensibility and APOE genotype).

Table 4. Association between arterial stiffness (pulse wave velocity (PWV) and carotid distensibility (CD)) and risk of dementia (hazard ratio (HR) and 95% confidence interval (CI) per standard deviation (SD) increase), using Cox' proportional hazard models

	PWV		CD	
	HR (95% CI)		HR (95% CI)	
	Model 1	Model 2	Model 1	Model 2
Per SD increase	0.97 (0.82-1.15)	0.91 (0.75-1.10)	1.08 (0.88-1.34)	1.05 (0.81-1.35)

Model 1: adjusted for age, sex and education

Model 2: additionally adjusted for mean arterial pressure, heart rate, current smoking, diabetes mellitus, body mass index, total cholesterol, high density lipid cholesterol and intima media thickness



Discussion

We did not find an association between arterial stiffness and cognitive decline or the risk of dementia. Though we found associations between arterial stiffness and several domains of cognitive function in cross-sectional analyses, these associations were small and after adjustment for mean arterial pressure, heart rate and cardiovascular risk factors only the association between increased PWV and poor performance on the Stroop test remained. Some aspects of the present study need to be discussed. Strengths of the Rotterdam Study are its population-based setting, its large number of persons and its virtually complete follow-up. A limitation of the study is that, since information on cognitive decline was only available for persons who participated in both the third and the fourth examination, selective attrition may have affected the results of our analyses regarding change in cognitive function. Persons included in these analyses were younger and had a better cardiovascular risk profile (including measures of arterial stiffness) than persons who did not participate in the fourth examination. Since age and cardiovascular factors are associated with cognitive function, this may have affected our power to find an association with cognitive decline. Another limitation is that the results of the analyses regarding cognitive decline may have been affected by regression to the mean. Regression to the mean may result in an underestimation of the association. However, follow-up was complete for the outcome dementia and we also found no relation between arterial stiffness and risk of dementia.

Few studies have examined the association between arterial stiffness and cognition. Recently, an association, independent of cardiovascular factors, between increased PWV and impaired cognitive function, defined by MMSE score, was found in patients who were referred to a memory clinic,⁶ and in community dwelling elderly.⁴ Our finding of an association independent of cardiovascular factors between increased PWV and worse performance on the Stroop test is in line with the notion that arterial stiffness affects cognitive function. However, we did not find independent associations between increased arterial stiffness and other cognitive tests. This may be explained by more extensive adjustments in our study compared to previous studies, including adjustments for carotid IMT, an indicator of atherosclerosis, body mass index, pulse rate and mean arterial pressure.

Few studies examined the association between arterial stiffness and dementia. In one study 308 elderly with complaints of memory loss were evaluated and classified in 4 groups (Alzheimer's disease, vascular dementia, mild cognitive impairment (MCI) and normal cognitive function).⁵ Persons with vascular dementia, AD and MCI had a higher PWV than those without cognitive impairment after adjustments for age, sex, systolic blood pressure, anti-hypertensive treatment and presence of cardiovascular diseases. In another study brachial-ankle PWV was compared between patients with AD, vascular dementia and cognitively normal age-matched controls. Arterial stiffness was higher in patients with vascular dementia than in those with AD or those without dementia.¹⁶ Another study reported an inverse correlation, adjusted for age, sex, mean arterial pressure and anti-hypertensive





treatment, between heart-brachial PWV and cognitive function, measured by the Hasegawa Dementia Scale Revised, in nonvascular dementia patients and persons with mild cognitive impairment.¹⁷

In our prospective study we did not find an association between arterial stiffness and risk of dementia or cognitive decline. However, we cannot completely rule out an association between increased arterial stiffness and risk of vascular dementia because of the low number of incident vascular dementia cases.

Arterial stiffness is strongly associated with hypertension and atherosclerosis that have both been related to an increased risk of dementia.^{2, 18-20} Therefore, an association of increased arterial stiffness with cognitive decline and dementia seemed plausible. Mechanisms for such an association include cerebrovascular disease (for instance, lacunar infarction or white matter lesions) and cerebral hypoperfusion. Though previous studies suggested that arterial stiffness might provide additional value above other cardiovascular risk factors in relation to cognitive decline or dementia, our data do not support this hypothesis.

To conclude, we failed to identify arterial stiffness as an independent risk factor of cognitive decline or risk of dementia.

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Chapter 3.4

Unrecognized myocardial infarction and the risk of dementia

Brief communication

Abstract

Vascular disease may play a role in the development of dementia. We examined the association between recognized and unrecognized myocardial infarction (MI) and risk of dementia in the Rotterdam Study, based on 613 dementia cases and 58,712 person-years of follow-up. Unrecognized MI increased the risk of dementia in men (adjusted hazard ratio, 95% CI: 2.19, 1.21-3.93), but not in women.

Introduction

Vascular disease and vascular risk factors are implicated in development of dementia, both vascular dementia and Alzheimer's disease.¹ Little is known about the association between myocardial infarction (MI) and dementia. An early prospective study among 488 elderly between ages 75 and 85, reported an association between a history of MI and a 5-fold increased risk of dementia in women, but not in men.² More recently, no association was found between dementia and preceding MI in the Rochester database.³

In the elderly, 33 to 54% of MI is clinically unrecognized,⁴ and only identified by EKG. We previously reported that unrecognized, but not recognized MI indicated an increased risk of stroke in men. In women, no association was found.⁵ Since both vascular factors and cerebrovascular disease are associated with risk of dementia, we hypothesized an association between MI and increased risk of dementia and examined this in the Rotterdam Study.

Methods

The Rotterdam Study is a population-based prospective cohort study among 7983 persons of 55 years and over.⁶ Baseline examinations (1990-1993) and follow-up examinations (1993-1994, 1997-1999 and 2002-2004) included physical examinations, screening and clinical work-up for dementia. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. Through linkage with records of general practitioners, the cohort was continuously monitored for morbidity and mortality. This resulted in a virtually complete follow-up until 2005.

At baseline, a 12-lead EKG was recorded with an ACTA-electrocardiograph (Esaote, Florence, Italy). All EKGs were processed by the Modular EKG Analysis System (MEANS).⁷ Based on a previously described procedure⁵ we classified persons as follows. The category of recognized MI included persons with a self-reported MI confirmed by EKG or clinical data. The unrecognized MI category consisted of persons without documented or self-reported MI, but with EKG matching an MI. The reference group (non-MI) consisted of persons without





indication of MI on EKG and no medical documentation of an earlier MI.

Of the 7,046 persons who were cognitively screened and not demented at baseline, EKG data were available for 6,347 persons. Missing data were mostly due to temporary technical difficulties with the EKG apparatus.

The diagnosis of dementia and subtypes of dementia was made following a three-step protocol as described previously.⁸ We used internationally accepted criteria for dementia (DSM-IIIIR), Alzheimer's disease (NINCDS-ADRDA) and vascular dementia (NINDS-AIREN).

The following factors were measured at baseline and considered as potential confounders.

Body mass index (BMI) (weight (kg)/height (m²)), smoking habits, blood pressure, total and high-density lipid (HDL) cholesterol, intima media thickness (IMT) measured by ultrasound, diabetes mellitus defined as a random or postload glucose level ≥ 11.1 mmol/l or use of blood glucose-lowering medication, and atrial fibrillation assessed with standard 12-lead EKGs. In addition, persons were categorized based on the presence or absence of an APOE $\epsilon 4$ allele.

Data analysis

We assessed the association of recognized and unrecognized MI with risk of dementia and subtypes of dementia with Cox' proportional hazard models, adjusted for age and sex. Additionally we adjusted for cardiovascular risk factors. Since we found an association between unrecognized MI and increased risk of stroke in men and not in women,⁵ we examined the association between MI and dementia in men and women separately and computed an interaction term between MI and sex. To assess whether the association between MI and dementia was mediated by stroke,⁵ we repeated the analyses excluding persons with prevalent stroke and censoring those with incident stroke at time of stroke.

Results

During 58,712 personyears of follow-up we identified 613 dementia patients, of whom 479 patients were diagnosed with Alzheimer's disease, 71 with vascular dementia and 63 with dementia due to other causes.

Recognized MI was identified at baseline in 424 participants (297 (70%) in men). Of the 345 unrecognized MI cases 159 (46.1%) were men.

Baseline characteristics of the study population are shown in table 1. Table 2 shows that recognized MI was not associated with risk of dementia. Unrecognized MI was associated with a more than doubled risk of dementia, but only in men. Additional adjustment for cardiovascular risk factors did not change the estimates. In men, unrecognized MI was associated with an increased risk of both Alzheimer's disease and vascular dementia. Age and sex adjusted hazard ratios (95% confidence interval (CI)) were 2.53 (1.49-4.30) for Alzheimer's disease (126 cases) and 2.03 (0.71-5.80) for vascular dementia (37 cases). The p-value of the interaction term was 0.01 between unrecognized MI and sex and 0.19 between



Table 1. Baseline characteristics of the study population (n=6,347)

	No myocardial infarction	Recognized myocardial infarction	Unrecognized myocardial infarction
N	5578	424	345
Age, yrs	68.3 (8.5)	71.2 (8.2)*	71.8 (8.8)*
Women %	61.4	30.0*	53.9*†
Presence APOE ε4 allele %	25.3	25.1	21.6
Body mass index, kg/m ²	26.3 (4.0)	26.4 (3.4)*	27.0 (4.5)*
Current smokers %	22.9	20.3	28.1*†
Systolic blood pressure, mmHg	139.1 (22.3)	135.2 (22.0)*	145.3 (20.6)*†
Diastolic blood pressure, mmHg	73.9 (11.4)	70.3 (11.3)*	75.4 (11.9)*†
Cholesterol, mmol/l	6.6 (1.2)	6.6 (1.2)*	6.6 (1.3)
HDL-cholesterol, mmol/l	1.4 (0.4)	1.1 (0.3)*	1.3 (0.3)*†
Intima media thickness, mm	0.78 (0.15)	0.85 (0.18)*	0.83 (0.16)*
Diabetes mellitus %	9.0	17.0*	13.0*†
Atrial fibrillation %	2.1	3.4	6.1*
Use of cardiovascular drugs %	33.0	83.0*	39.0†

Values represent means (SD) or percentages

* Significant difference (p-value <0.05) compared to persons without MI (when applicable, age and sex adjusted)

† Significant difference (p-value <0.05) compared to recognized MI (when applicable, age and sex adjusted)

recognized MI and sex.

Excluding previous stroke cases and censoring incident stroke cases at time of stroke did not change the estimates; if anything the association became stronger. The age and sex adjusted hazard ratio (95% CI) for dementia in men with unrecognized MI was 2.33 (1.38-3.95)

Discussion

We found that unrecognized MI indicated a strongly increased risk of dementia in men, but not in women. Recognized MI was not associated with risk of dementia in either sex. The association was independent of cardiovascular risk factors and could not be explained by the occurrence of clinical stroke.

The strengths of our study include its prospective design, the population-based setting, large number of participants, and virtually complete follow-up.

Table 2. The association between MI (recognized and unrecognized) and dementia by sex. Hazard ratio and 95% confidence interval

	Total (613 cases)		Men (199 cases)		Women (414 cases)	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
No MI	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Recognized MI	1.06 (0.78-1.46)	1.12 (0.77-1.64)	0.87 (0.55-1.39)	0.89 (0.51-1.54)	1.35 (0.88-2.06)	1.58 (0.94-2.65)
Unrecognized MI	1.22 (0.90-1.65)	1.35 (0.95-1.92)	2.14 (1.37-3.35)	2.23 (1.24-4.01)	0.87 (0.58-1.32)	1.17 (0.74-1.83)

Model 1: adjusted for age and sex (when applicable)

Model 2: additionally adjusted for presence of APOE ε4 allele, systolic blood pressure, diastolic blood pressure, body mass index, atrial fibrillation, diabetes mellitus, current smoking, intima media thickness, total cholesterol and high-density lipid cholesterol.



A possible limitation of the study is that MI on EKG was diagnosed using the MEANS computer program, which might have led to misclassification. However, this program has been extensively validated and diagnoses correlate well with diagnoses made by an experienced cardiologist.⁷ Also, any misclassification is likely to be non-differential since MEANS diagnoses were made independent from and before clinical diagnosis of dementia. Few studies have reported on the association between MI and dementia. In a prospective study in the very old an association between history of MI and dementia was found in women only. The interaction term between history of MI and sex was significant.² More recently, the association between MI and dementia was examined using a record-linkage system to identify dementia cases and matched controls. The odds ratio (95% CI) for MI before dementia diagnosis among cases with dementia compared with controls was 1.00 (0.62-1.62).³ In accordance with our findings of stroke risk associated with MI, we found that men with unrecognized but not with recognized MI had an increased risk of dementia. How can we explain these differences in risk associated with unrecognized and recognized MI? It has been suggested that persons with unrecognized MI are older, more often diabetic and have higher blood pressure compared with persons with recognized MI.^{9,10} However, adjustment for these risk factors did not change the association. Another explanation might be that men with unrecognized MI are less often treated with cardiovascular drugs and less often quit smoking (table 1).

The sex differences in risk associated with unrecognized MI fit previous observations regarding risk of cardiovascular death⁹ and stroke.⁵ However, it remains unclear what explains these differences. It has been suggested that misclassification of unrecognized MI occurs more often in women. This may have resulted in an underestimation of the association with dementia in women.

Though we previously found an association between unrecognized MI and stroke,⁵ taking into account stroke in the analyses did not change the association, suggesting that other mechanisms -at least partly- play a role. Possibly, cerebral small vessel disease explains the association. Though replication of our findings is needed, our study suggests that coronary heart disease is associated with an increased risk of dementia.

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Chapter 4. Markers for early detection of dementia

Chapter 4.1

Subjective memory complaints, education and risk of Alzheimer's disease

Abstract

Background Subjective memory complaints are common in the elderly. Although memory complaints are associated with an increased risk of Alzheimer's disease in persons with cognitive impairment as well as in persons with normal cognition they are commonly considered of less importance than objective cognitive measures. We hypothesized that the clinical relevance of subjective memory complaints might vary with educational background.

Methods The study was performed within the Rotterdam Study, a prospective population-based cohort study among 7,983 persons of 55 years and over. Subjective memory complaints and level of education were assessed in the baseline interview (1990-1993). During a mean follow-up of 9.0 years we identified 568 incident Alzheimer's disease patients. We estimated the association between subjective memory complaints and risk of dementia by means of Cox' proportional hazard models.

Results The association between subjective memory complaints and risk of Alzheimer's disease varied across levels of education. The risk of Alzheimer's disease associated with subjective memory complaints was higher in highly educated persons (age and sex adjusted hazard ratio 2.33, 95% CI 1.00-5.49) than in persons with a low education (age and sex adjusted hazard ratio 1.53, 95% CI 1.15-2.05) (p-value for interaction 0.02). In highly educated persons without objective cognitive impairment (MMSE score 29 or 30) the risk of Alzheimer's disease was highest (age and sex adjusted hazard ratio 2.98, 95% CI 1.76-5.02).

Conclusions Especially in persons with a high level of education who still perform well on formal cognitive tests, subjective memory complaints may be an important first sign of imminent Alzheimer's disease.

Introduction

Subjective memory complaints are common in the elderly. The clinical relevance of subjective memory complaints in predicting future dementia, predominantly Alzheimer's disease, is unclear. Several prospective population-based studies have found an association between subjective memory complaints and risk of dementia,^{1,2} and over the years awareness has increased that persons who complain about their memory should be taken seriously and should be examined for dementia. As a result, general practitioners have become more inclined to



administer brief cognitive screening tests to people that complain about their memory. However, the question is whether cognitive screening tests are sufficient to detect early Alzheimer's disease especially in persons with a higher level of education. Subtle cognitive deficits in highly educated persons might be overlooked due to a ceiling effect of screening tests. Only few studies have reported an association between subjective memory complaints and the risk of dementia in persons with normal cognitive function at baseline.^{3, 4} It has been suggested that the association between memory complaints and dementia in persons with normal baseline cognition is driven by highly educated persons.² An association between subjective memory complaints and Alzheimer's disease is plausible, yet has not been properly investigated in persons who perform well on screening tests and the modifying effect of education has not been quantified. The objective of the present study was to examine whether the association between subjective memory complaints and the risk of dementia varied across levels of education. We performed this study in the Rotterdam Study, a prospective population-based cohort study among 7,983 persons of 55 years and over.

Methods

Study population

The Rotterdam Study is a population-based prospective cohort study that investigates the incidence and risk factors of cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases in the elderly.⁵ From 1990-1993, all 10,275 residents aged 55 years or over of Ommoord, a district of the city of Rotterdam, The Netherlands, were invited to participate in an extensive home interview and two visits to the research center, and 7,983 (78%) of them agreed. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants. Follow-up examinations were conducted in 1993-1994, 1997-1999 and 2002-2004. In addition, through linkage with records of general practitioners and the municipality, the total cohort was continuously monitored for morbidity and mortality.

Of all participants who had undergone cognitive screening and were not demented at baseline (n=7,046), we had data on subjective memory complaints of 6,927 persons.

Assessment of subjective memory complaints and education

During 1990-1993, trained investigators interviewed all participants at home. The presence of subjective memory complaints was assessed by the question "Do you have memory complaints?". Information on highest education obtained was collected. We categorized level of education in four groups. The first group contained persons with primary education (low education), the second group combined persons with primary education plus a higher not completed education with persons with lower vocational education (low-intermediate education), the third group combined persons with intermediate vocational education with persons with general secondary



education (high-intermediate education), and the fourth group contained persons with higher vocational education or university training (high education).

Diagnosis of dementia

The diagnosis of dementia was made following a three-step protocol.^{6,7} Two brief tests of cognition (Mini-Mental State Examination (MMSE) and Geriatric Mental State schedule (GMS) organic level) were used to screen all subjects. Screen-positives (MMSE score <26 or GMS organic level >0) underwent the Cambridge examination for mental disorders of the elderly (Camdex). Persons who were suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for diagnosis. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Healthcare. The diagnosis of dementia and subtype of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III-R), Alzheimer's disease (NINCDS-ADRD), and vascular dementia (NINDS-AIREN) by a panel of a neurologist, neuropsychologist and research physician.

Covariates

Information on periods of depression and treatment of depressive symptoms was obtained in the baseline interview. At the research center, clinical measures were obtained. Glucose was measured in non-fasting blood samples. The body mass index (BMI) was calculated (weight (kg)/length (m²)). Genotyping for APOE was performed on coded DNA specimens. Furthermore, ultrasonography of both carotid arteries was performed. As an indicator of atherosclerosis of the carotid arteries, we used intima media thickness (IMT). Common carotid IMT was determined as the average of the maximum IMT of near-and far-wall measurements, and the average of left and right common carotid IMT was computed.⁸ We defined diabetes mellitus as a random or postload glucose level ≥ 11.1 mmol/L or the use of blood glucose-lowering medication.

Data analysis

We investigated the association between level of education and the risk of Alzheimer's disease in our population by means of Cox' proportional hazard models, adjusted for age and sex. Because vascular risk factors may be associated with both education level and risk of Alzheimer's disease, we additionally adjusted for current smoking, BMI, carotid IMT and diabetes mellitus in the analyses.

We examined the association between subjective memory complaints and risk of Alzheimer's disease by means of Cox' proportional hazard models, adjusted for age and sex. Additionally we adjusted for the presence of an APOE $\epsilon 4$ allele, level of education and baseline MMSE score.



To assess the temporal relationship between subjective memory complaints and AD, we performed separate analyses using incident cases identified until 2000 (short follow-up, mean 5.7 years since baseline) and those identified between 2000 and 2004 (long follow-up, mean 10.8 years since baseline).

Because persons with depressive symptoms frequently report memory complaints and depressive symptoms are common in Alzheimer's disease, we repeated the analyses excluding persons who had reported periods of depression in the baseline interview. To test our hypothesis that the association between subjective memory complaints and risk of Alzheimer's disease varies across level of education, we repeated the analyses stratified on education level. Also, an interaction term between subjective memory complaints and level of education was computed. In subsequent analyses we examined the association between subjective memory complaints and risk of Alzheimer's disease across levels of education, using persons with low education without subjective memory complaints as the reference category.

Next, since we considered that the association between subjective memory complaints and Alzheimer's disease in persons with normal cognition might be driven by highly educated persons, we examined the association between subjective memory complaints and risk of Alzheimer's disease across different scores of the baseline MMSE, stratified on level of education, using persons with low education without subjective memory complaints as the reference category. Because of low numbers, we combined the two lowest (low) and the two highest (high) education levels for these analyses. We computed an interaction term between subjective memory complaints, level of education and MMSE score at baseline.

Results

The baseline characteristics of the total study population and across persons with and without subjective memory complaints are shown in table 1.

Table 2 shows that both men and women with higher level of education had a lower risk of Alzheimer's disease, though the association seemed strongest in men.

Compared to persons with low education (reference) persons with highest level of education had a 40% decreased risk of Alzheimer's disease (age and sex adjusted hazard ratio (HR) (95% confidence interval (CI)) 0.60 (0.39-0.93)). This estimate did not change markedly after adjustment for vascular factors (HR (95% CI) 0.56 (0.31-0.99)).

Subjective memory complaints were associated with an increased risk of Alzheimer's disease (table 3). Additional adjustment for presence of an APOE $\epsilon 4$ allele, level of education and baseline MMSE score did not change the association markedly (HR (95% CI)) for Alzheimer's disease 1.71 (1.41-2.08)). The risk of Alzheimer's disease was still increased when we confined the analyses to incident cases detected after the year 2000 (age and sex adjusted HR (95% CI) using incident cases before 2000 was 2.07 (1.62-2.64) and 1.66 (1.28-2.15) when using only cases identified after 2000.



Table 1. Baseline characteristics of the study population (n=6927) across persons with (n=1309) and without (n=5618) subjective memory complaints

	Total group (n=6927)	With memory complaints (n=1309)	Without memory complaints (n=5618)	P-value difference [¶]
Age, years (SD*)	69.5 (9.1)	71.6 (9.4)	68.9 (8.8)	<0.001
Female sex N (%)	4132 (60.0)	818 (62.5)	3314 (59.0)	0.421
APOE ε4 allele N (%)	1601 (25.0)	322 (26.8)	1279 (24.6)	0.002
Baseline MMSE score (SD)	27.6 (1.9)	27.2 (2.1)	27.7 (1.8)	<0.001
Current smoking N (%)	1565 (22.8)	256 (20.0)	1309 (23.5)	0.317
Intima media thickness, mm (SD)	0.79 (0.16)	0.80 (0.16)	0.79 (0.16)	0.910
Diabetes mellitus N (%)	690 (10.0)	137 (10.5)	553 (9.9)	0.724
Body mass index, kg/m ² (SD)	26.3 (4.0)	26.2 (3.8)	26.4 (4.0)	0.006
Memory complaints N (%)	1309 (18.9)	n/a	n/a	
Education level N (%)				<0.001
Low	1640 (24.1)	380 (29.9)	1260 (22.7)	
Low-intermediate	2060 (30.3)	377 (29.7)	1683 (30.4)	
High-intermediate	2541 (37.3)	433 (34.1)	2108 (38.1)	
High	567 (8.3)	79 (6.2)	488 (8.8)	

* SD=standard deviation

¶ Adjusted for age and sex when applicable

Exclusion of persons with self-reported periods of depression (n=2204) did not change the estimates (age and sex adjusted HR (95% CI) for Alzheimer's disease was 2.17 (1.70-2.77). The relationship between risk of Alzheimer's disease and subjective memory complaints varied across levels of education. In persons with a higher level of education, the association between subjective memory complaints and Alzheimer's disease was strongest (table 3). The p-value of the interaction term between subjective memory complaints and education level was 0.02. Compared with persons with low education without subjective memory complaints the risk of Alzheimer's disease associated with subjective memory complaints was similar across levels of education (figure 1). Figure 2 shows that the association between subjective memory complaints and risk of Alzheimer's disease was especially strong in persons with a higher education who performed well on the MMSE (hazard ratio (95% confidence interval) for dementia 2.98 (1.76-5.02)).

Table 2. The association of education and risk of Alzheimer's disease by sex (hazard ratio (HR) and 95% confidence interval (CI))

Education level	HR (95% CI) Total (533 cases)		HR (95% CI) Men (137 cases)		HR (95% CI) Women (396 cases)	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Low	1.00	1.00	1.00	1.00	1.00	1.00
Low-intermediate	0.87 (0.70-1.08)	0.82 (0.63-1.07)	0.86 (0.53-1.37)	0.70 (0.38-1.28)	0.89 (0.69-1.10)	0.84 (0.63-1.13)
High-intermediate	0.76 (0.61-0.95)	0.72 (0.54-0.95)	0.75 (0.49-1.13)	0.65 (0.39-1.08)	0.75 (0.57-0.98)	0.73 (0.52-1.02)
High	0.60 (0.39-0.93)	0.56 (0.31-0.99)	0.39 (0.18-0.84)	0.30 (0.10-0.86)	0.83 (0.49-1.41)	0.86 (0.44-1.70)
Ptrend	0.003	0.006	0.017	0.017	0.048	0.091

Model 1: Adjusted for age and sex

Model 2: Additionally adjusted for body mass index, ever smoking, diabetes mellitus, intima media thickness and presence of APOE ε4 allele

Table 3. Subjective memory complaints and the risk of Alzheimer's disease (hazard ratio (HR) and 95% confidence interval (CI))* in the total group and stratified on education level

	N (cases)	HR (95% CI)
Total group	6927 (568)	1.87 (1.57-2.24)
Stratified on education		
Low education	1993 (215)	1.53 (1.15-2.05)
Low-intermediate education	2250 (156)	1.90 (1.35-2.68)
High-intermediate education	2708 (143)	2.26 (1.59-3.21)
High education	618 (25)	2.33 (1.00-5.49)

*Adjusted for age and sex

The p-value of the interaction term between subjective memory complaints, level of education and MMSE score at baseline was 0.008.

Discussion

We found that persons who complain about their memory had an increased risk of Alzheimer's disease. This association varied across level of education, being strongest in persons with a high level of education. Subjective memory complaints were associated with an increased risk of Alzheimer's disease particularly in highly educated persons who performed well on the baseline MMSE.

The strengths of the Rotterdam Study are its prospective design, the population-based setting, its large number of subjects, and its virtually complete follow-up. A limitation is that no clinical assessment of depression and depressive symptoms at baseline was available. Persons with depressive symptoms frequently report memory complaints and depressive symptoms are common in early stage Alzheimer's disease. Consistent with reports on the life-time prevalence on depression, a considerable number of persons in our study reported to have experienced periods of depression (n=2204). By considering all these persons as possibly depressed at baseline we may have overestimated the amount of persons who were clinically depressed. Still, excluding these persons from the analyses did not change the association. Hence we are confident that depressive symptoms did not explain the association between subjective memory complaints and Alzheimer's disease.

We assessed subjective memory complaints by means of a single question.

Cognitive function at baseline was assessed by means of the MMSE that is thought to be a non-sensitive screening tool for cognitive impairment, though widely used in daily clinical practice.



Subtle cognitive in highly educated persons is likely missed due to ceiling effects, as is suggested by our finding that subjective memory complaints were most strongly associated with Alzheimer's disease in highly educated persons that still performed well on the MMSE. An alternative, not mutually exclusive, explanation is that highly educated people may be more noticeable of subtle changes in their own performance. Unfortunately, more extensive cognitive testing, that might have shown these subtle cognitive deficits in higher educated persons, was not available in our study.

Several population-based studies have examined the association of subjective memory complaints with risk of dementia and cognitive decline.^{1, 2, 4, 9} Memory complaints predicted dementia in elderly persons with cognitive impairment¹⁰ as well as in elderly persons with normal cognition.^{3, 4} Geerlings et al. found an association between subjective memory complaints and risk of dementia in persons with normal baseline cognition, assessed by means of MMSE. In that study, the absence of an association in persons with impaired cognition can be explained by the high percentage of loss to follow-up in this group,⁴ which might have made it more difficult to find an association. We found an association between subjective memory complaints and an increased risk of Alzheimer's disease across all baseline MMSE scores.

The association between education and risk of dementia has been widely studied. Most studies have found that high education protected against dementia.¹¹⁻¹³ We found a similar association in our study.

The role of education in relation to the association between subjective memory complaints and risk of dementia is unclear. The study by Geerlings et al. reported no interaction between memory complaints and level of education, defined as high (>8 years) or low (≤ 8 years), in relation to the risk of dementia. We reported a significant interaction between subjective memory complaints and level of education. Though persons with a high education had a lower risk of Alzheimer's disease, the risk associated with subjective memory complaints is similar to that of persons with a low education. In persons who performed well on the baseline MMSE, the risk of Alzheimer's disease associated with subjective memory complaints was much higher in highly educated persons than in persons with a low education. The strong association between subjective memory complaints and Alzheimer's disease especially in highly educated persons without objective cognitive impairment is suggestive of an under-detection of early stage dementia in these persons. Though we were not able to examine this selective under-detection because of lack of more extensive neuropsychological tests at baseline, possibly, these persons experience cognitive deficits related to early stage dementia, not detected by MMSE, and therefore complain about their memory. These findings emphasize the importance of self-perceived cognitive deterioration, expressed as subjective memory complaints in highly educated persons.

Our study suggests that brief cognitive screening tests may be insufficient to detect early signs of Alzheimer's disease in highly educated persons with subjective memory complaints.



Figure 1. The association between subjective memory complaints and risk of Alzheimer's disease, stratified on level of education, using persons with low education and no subjective memory complaints as the reference category (R) (hazard ratio (HR) and 95% confidence interval)

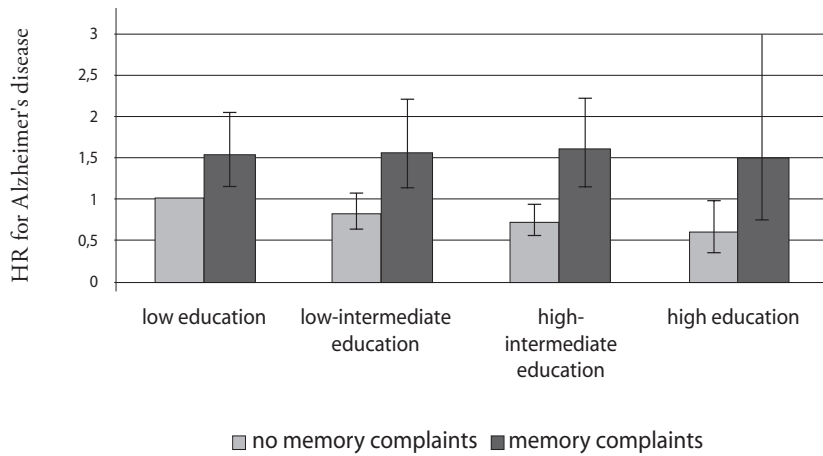
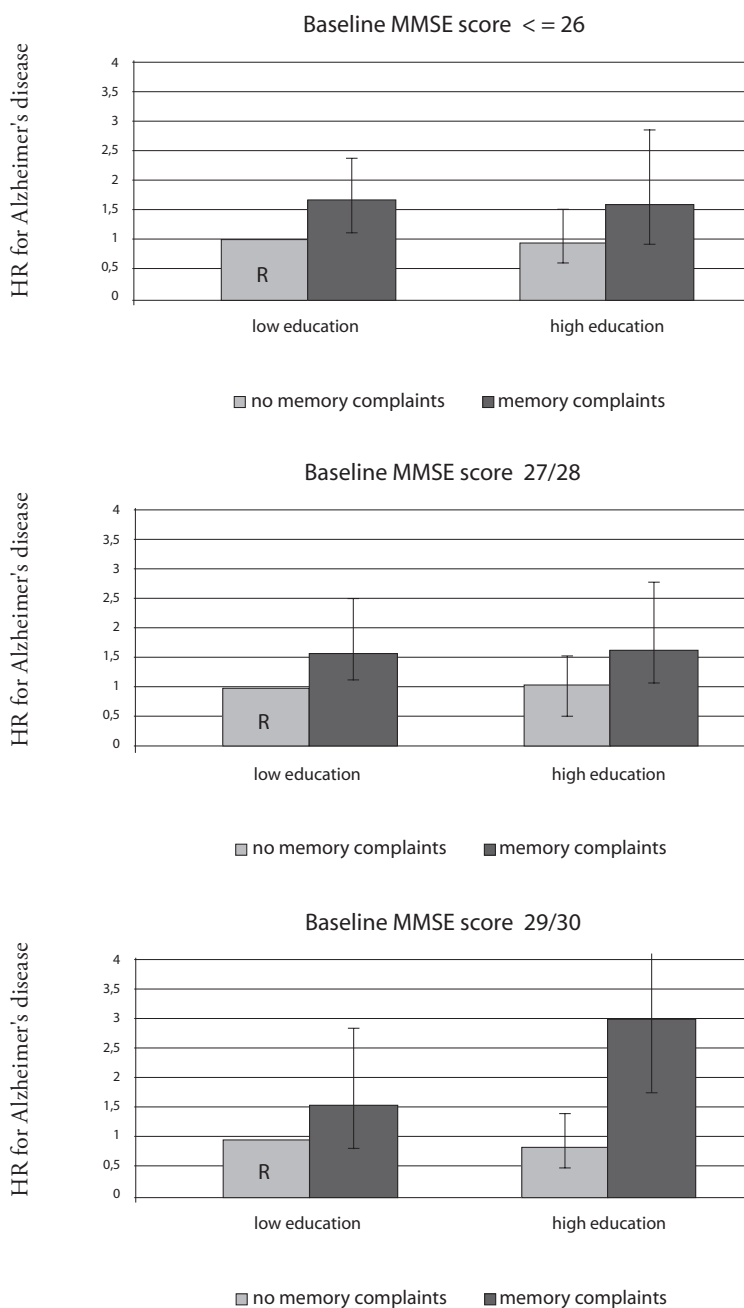


Figure 2. The association between subjective memory complaints and risk of Alzheimer's disease across baseline MMSE scores, stratified on level of education (low and low-intermediate education levels (low) and high-intermediate and high education levels (high) were combined), using persons with low education and no subjective memory complaints as the reference category (R) (hazard ratio (HR) and 95% confidence interval)



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Chapter 4.2

Plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ and the risk of dementia

Abstract

Background Amyloid β -peptides ($A\beta$), $A\beta_{1-40}$ and $A\beta_{1-42}$, are important components of plaques in Alzheimer's disease. Their plasma levels increase with age and are increased in persons with mutations that cause early-onset Alzheimer's disease, yet $A\beta_{1-42}$ may decrease early in the dementia process. We hypothesized an association of levels of plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ with risk of dementia.

Methods We performed a case-cohort study embedded in the prospective, population-based Rotterdam Study. Of 6,713 participants at risk for dementia, a random sample of 1,756 persons was drawn. During follow-up (mean 8.6 years), 392 incident dementia cases were identified. We investigated the association between plasma $A\beta$ levels and risk of dementia and subtypes of dementia, with Cox' proportional hazard models.

Findings High baseline $A\beta_{1-40}$ but not $A\beta_{1-42}$ was associated with an increased risk of dementia. Compared to the first quartile of $A\beta_{1-40}$, age and sex adjusted hazard ratios (95% confidence interval) for dementia for the second, third and fourth quartile were 1.07 (0.72-1.58), 1.16 (0.78-1.70) and 1.46 (1.01-2.12) (Ptrend 0.03). Persons with an increased $A\beta_{1-42}/A\beta_{1-40}$ ratio had a lower risk of dementia. Compared to the first quartile of the $A\beta_{1-42}/A\beta_{1-40}$ ratio, hazard ratios (95% confidence interval) for the second, third and fourth quartiles were 0.74 (0.53-1.02), 0.62 (0.44-0.88) and 0.47 (0.33-0.67) (Ptrend < 0.001). Associations were similar for Alzheimer's disease and vascular dementia.

Interpretation High plasma levels of $A\beta_{1-40}$ especially when combined with low levels of $A\beta_{1-42}$ indicate an increased risk of dementia. A potential role of plasma $A\beta$ levels as a marker of incipient dementia warrants further investigation.

Introduction

The presence of amyloid-containing senile plaques together with neurofibrillary tangles is a hallmark of Alzheimer's disease (AD).¹ Important components of these plaques are amyloid β_{1-40} ($A\beta_{1-40}$) and amyloid β_{1-42} ($A\beta_{1-42}$), derived from the β -amyloid precursor protein (β APP).² Previous reports suggest that in AD brains, $A\beta_{1-42}$ is deposited first and constitutes the predominant form in senile plaques, whereas $A\beta_{1-40}$ is deposited later in the disease.^{3,4} It is not clear how $A\beta$ deposition in the brain affects plasma $A\beta$. A complex equilibrium exists between brain production and deposition^{5,6} of $A\beta$ as well as peripheral production by platelets.⁷

Both plasma $A\beta_{1-40}$ and plasma $A\beta_{1-42}$ increase with age over 65 years.^{8,9} Plasma levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ are increased in persons who carry mutations that cause early onset familial AD¹⁰ and in patients with Down's syndrome who are at increased risk of developing AD.¹¹ Also, plasma $A\beta$ is increased in first degree AD relatives, who are at a higher risk of developing AD.¹²



In a previous longitudinal study, higher baseline plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ levels were found in persons who developed dementia 5 years later compared to persons who remained free of dementia, suggesting that increased plasma levels of $A\beta$ are involved in the development of dementia.⁹ In the same study, it was found that plasma $A\beta_{1-42}$ decreased over time with newly acquired Alzheimer's disease. Other studies provide evidence for a decline in plasma $A\beta_{1-42}$ in the presymptomatic period of AD. Graff-Radford et al reported that plasma $A\beta_{1-42}$ declines at an average rate of 12%/year in subjects with mild cognitive impairment whereas plasma $A\beta$ rises at an average rate of 9%/year in non-demented controls.¹³ A recent longitudinal study showed that higher baseline plasma $A\beta_{1-42}$ levels and greater reductions in $A\beta_{1-42}$ levels were associated with reductions in cognitive scores.¹⁴ An explanation for this selective decrease may be that both CSF and plasma $A\beta_{1-42}$ decrease when $A\beta_{1-42}$ is deposited in the brain.¹⁵ The relation of plasma $A\beta_{1-42}$ levels with risk of dementia may therefore vary with time to onset. We examined whether plasma levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ were associated with risk of dementia and subtypes of dementia.

We investigated this association in the Rotterdam Study, a prospective population-based cohort study among men and women aged 55 years and over.

Methods

Study population

The Rotterdam Study is a prospective population-based cohort study that investigates the incidence and risk factors of cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases in the elderly.¹⁶ From 1990-1993, all 10,275 residents aged 55 years or over of Ommoord, a district of the city of Rotterdam, were invited to participate in an extensive home interview and two visits to the research center, and 7,983 (78%) of them agreed. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all participants.

At the baseline clinical examination, blood samples were drawn from 7,050 persons of whom 7,047 underwent screening for dementia. In these, prevalent dementia was diagnosed in 334, resulting in a cohort at risk for dementia of 6,713 persons.

Follow-up examinations were conducted in 1993-1994, 1997-1999 and 2002-2004. In addition, through linkage with records of general practitioners and the municipality, the total cohort was continuously monitored for morbidity and mortality. This resulted in a virtually complete follow-up until January 1, 2005.

Case-cohort analysis

For reasons of efficiency a case-cohort design was used.^{17,18} In this, a random sample, or a "subcohort" is drawn from the source population which constitutes the reference group. Cases who develop outside the subcohort but within the cohort at risk are added to the analyses, yet



only persons from the subcohort contribute follow-up time. In our study the source population consisted of 6,713 persons at risk of dementia, who were followed from baseline (1990-1993) until the end of 2004 for incident dementia. From this source population we drew a random subcohort of 1,756 persons. In the subcohort, 162 persons developed dementia during follow-up. To this, we added 230 patients who developed dementia during follow-up outside the subcohort. In total, we therefore included 392 incident dementia cases in the analyses.

Measurement of plasma $A\beta_{1-40}$ and $A\beta_{1-42}$

At baseline, non-fasting blood samples were collected into vacutainers containing sodium citrate. These samples were put on ice immediately and centrifuged within 60 minutes, and aliquots of plasma were stored at -80 degrees Celcius. Plasma levels of amyloid β were determined by a double-antibody sandwich enzyme-linked immunosorbent assay method (Pfizer, Ann Arbor, MI, USA). The assay is based on a DELFIA immunoassay developed by Pfizer. A 96-well sandwich ELISA format was utilized. In brief, low fluorescence Delfia microtiter plates were coated with 100ul of 5ug/ml of 6E10 in carbonate buffer (pH 9.9 – Pierce 28382) for 1 hr at room temperature, then overnight at 4C. Plates were allowed to come to room temperature, then washed with DELFIA wash buffer (Perkin-Elmer). Plates were then blocked with 2% BSA and 0.2% Tween-20 in PBS for 1 hour. Following blocking step, plates were washed and then 125ul ($A\beta_{1-42}$) or 100ul ($A\beta_{1-40}$) of QCs standards or samples were added. Protease inhibitors were added to samples prior to analysis (Roche 1836145). Plates were incubated overnight at 4C. Following overnight incubation, plates were allowed to come to room temperature and washed and then 100ul of R226 ($A\beta_{1-42}$; 1:400) or R209 $A\beta_{1-40}$ (1:1000) were added and plates incubated at room temperature for 90 minutes. Plates were then washed and 100ul of Europium labeled streptavidin added per manufacturer's instructions (Perkin-Elmer). Plates were incubated in dark for 1hr, washed and signal enhancement per manufacturer's instructions. Plates were read utilizing time resolved flourometry from a Wallac Model 1420 Multi-label counter (Victor2) instrument. Assays performed well with sodium citrate and EDTA plasma. Heparin is not recommended as a coagulant as heparin binds to Abeta and significantly quenches signal. Aliquots of pooled patient samples with varying ranges of Abeta (low, medium and high) were frozen and utilized as quality control (QC) samples. Abeta peptide standards were obtained from California Peptide and stored in DMSO at -80 C. The mean coefficients of within and between assays variations were 4.4% and 10.1% for $A\beta_{1-40}$, and 4.9% and 14.8% for $A\beta_{1-42}$. The detection limits were 10 to 1,000 pg/ml for $A\beta_{1-40}$ and 5 to 100 pg/ml for $A\beta_{1-42}$.

Diagnosis of dementia

At baseline and during follow-up examinations the diagnosis of dementia followed a similar three-step protocol. Two brief tests of cognition (Mini-Mental State Examination (MMSE) and Geriatric Mental State schedule (GMS) organic level) were used to screen all subjects. Screen-



positives (MMSE score < 26 or GMS organic level > 0) underwent the Cambridge examination for mental disorders of the elderly (Camdex). Persons who were suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for diagnosis. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. The diagnosis of dementia and subtype of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III-R), Alzheimer's disease (NINCDS-ADRDA)¹⁹ and vascular dementia (NINDS-AIREN)²⁰ by a panel of a neurologist, neuropsychologist and research physician.

Table 1. Baseline characteristics of the random cohort (n=1,756) and the total cohort at risk (n=6,713)

Variable	Total cohort at risk	Random cohort
Age (years) (SD*)	69.5 (9.1)	68.6 (8.6)
Women (%)	59.9	61.0
APOE ε4 present (%)	25.1	24.5
Body Mass Index (kg/m ²) (SD)	26.3 (4.0)	26.2 (3.6)
Total cholesterol (mmol/l) (SD)	6.6 (1.2)	6.7 (1.2)
HDL cholesterol (mmol/l) (SD)	1.3 (0.4)	1.3 (0.4)
Creatinine (mmol/l)**	80.0 (72.0-91.0)	80.0 (71.0-91.0)
Diabetes mellitus (%)	10.0	9.0
Aβ ₁₋₄₀ pg/ml**	-	192.0 (163.0-228.0)
Aβ ₁₋₄₂ pg/ml**	-	17.8 (14.7-21.7)
Aβ ₁₋₄₂ /Aβ ₁₋₄₀ pg/ml**	-	0.093 (0.081-0.106)

* SD=standard deviation

** Levels are presented as median (interquartile range)

Covariates

At baseline a trained investigator visited all participants at home and collected information by means of a computerized questionnaire. The interview included current health status, medical history, drug use, and smoking behavior. Additionally, during two visits to the research center, clinical measures were obtained. Height and weight were measured and the body mass index (BMI) was calculated (weight (kg)/height (m²)). Non-fasting blood samples were drawn and immediately frozen. Total cholesterol and high density lipid (HDL) -cholesterol were measured within 2 weeks. Genotyping for APOE was performed on coded DNA specimens without

Table 2. The association between plasma A β and risk of dementia. Hazard ratio (95% confidence interval) for dementia (392 cases)

	A β ₁₋₄₀ Model 1	Model 2	A β ₁₋₄₂ Model 1	Model 2	A β ₁₋₄₂ /A β ₁₋₄₀ Model 1	Model 2
Per SD increase	1.17 (1.04-1.33)	1.17 (1.02-1.33)	0.88 (0.71-1.10)	0.90 (0.72-1.12)	0.58 (0.42-0.80)	0.63 (0.45-0.87)
1 st quartile	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
2 nd quartile	1.07 (0.72-1.58)	0.99 (0.67-1.47)	0.89 (0.62-1.27)	0.86 (0.60-1.25)	0.74 (0.53-1.02)	0.77 (0.55-1.08)
3 rd quartile	1.16 (0.78-1.70)	1.09 (0.73-1.62)	0.77 (0.54-1.10)	0.78 (0.54-1.14)	0.62 (0.44-0.88)	0.66 (0.46-0.96)
4 th quartile	1.46 (1.01-2.12)	1.32 (0.90-1.96)	0.86 (0.61-1.22)	0.82 (0.57-1.19)	0.47 (0.33-0.67)	0.53 (0.36-0.76)
Ptrend	0.03	0.01	0.32	0.28	<0.001	<0.001

Model 1: Adjusted for age and sex

Model 2: Adjusted for age, sex, presence of APOE ϵ 4 allele, creatinine, total cholesterol, high-density lipid-cholesterol and body mass index

knowledge of diagnosis. Two groups were formed on the basis of presence or absence of an APOE $\epsilon 4$ allele. Plasma creatinine levels were assessed using an automated enzymatic procedure (Roche, Mannheim, Germany). We defined diabetes mellitus as a random or postload glucose level ≥ 11.1 mmol/l or the use of blood glucose lowering medication.

Data analysis

The correlation of potential confounders with plasma $A\beta_{1-40}$, $A\beta_{1-42}$ and the ratio $A\beta_{1-42}/A\beta_{1-40}$ in the random subcohort was examined.

We examined the association of plasma levels $A\beta_{1-40}$, $A\beta_{1-42}$ and the ratio $A\beta_{1-42}/A\beta_{1-40}$ with risk of dementia using standard Cox' proportional hazard models with modification of the standard errors based on robust variance estimates. We used the method according to Barlow in which the random cohort is weighted by the inverse of the sampling fraction from the source population.^{17,18}

The proportional hazards assumption was tested by adding interaction terms of $A\beta_{1-40}$, $A\beta_{1-42}$ and the ratio $A\beta_{1-42}/A\beta_{1-40}$ with follow-up time in the different models.

We examined the association of $A\beta_{1-40}$, $A\beta_{1-42}$ and the ratio $A\beta_{1-42}/A\beta_{1-40}$ with the risk of dementia and subtypes of dementia entering $A\beta_{1-40}$, $A\beta_{1-42}$ and the ratio as linear terms (per standard deviation (SD)) in the models. Then, quartiles of $A\beta_{1-40}$, $A\beta_{1-42}$ and the ratio $A\beta_{1-42}/A\beta_{1-40}$ were made and the lowest quartiles were used as reference categories. Analyses were adjusted for age, sex and subsequently for other potential confounders including presence of the APOE $\epsilon 4$ allele, serum creatinine levels, total cholesterol, HDL-cholesterol and BMI. We investigated whether the association between plasma $A\beta$ and dementia was different in carriers and non-carriers of the APOE $\epsilon 4$ allele and in persons with and without diabetes mellitus. We also examined the association of plasma $A\beta_{1-40}$ with dementia across tertiles of plasma $A\beta_{1-42}$ levels. Analyses were performed using SAS 8.2 and SPSS 11.0 statistical software.

Results

Baseline characteristics of the cohort at risk and the random cohort are shown in table 1. Of the total of 392 incident dementia patients, 289 were diagnosed with Alzheimer's disease (AD), of which 31 with Alzheimer's disease with cerebrovascular disease; 54 with vascular dementia; 17 with dementia in Parkinson's disease and 32 patients with dementia due to other causes such as multisystem atrophy, frontotemporal dementia and Lewy body dementia.

None of the interaction terms of $A\beta_{1-40}$, $A\beta_{1-42}$ and the ratio $A\beta_{1-42}/A\beta_{1-40}$ with follow-up time was significant. Table 2 shows that increasing levels of plasma $A\beta_{1-40}$ but not plasma $A\beta_{1-42}$ were associated with an increased risk of dementia. An increased ratio $A\beta_{1-42}/A\beta_{1-40}$ was associated with a lower risk of dementia. Persons in the upper quartile had a 53% lower risk of dementia compared to persons in the lowest category (hazard ratio (HR) 0.47, 95% confidence interval (CI) 0.33-0.67). Additional adjustment for serum creatinine did not markedly affect the estimates, nor did additional adjustment for presence of APOE $\epsilon 4$ allele, total cholesterol, HDL-cholesterol and

BMI (HR 0.53, 95% CI 0.36-0.76). Table 3 shows that the age and sex adjusted hazard ratios for Alzheimer's disease and vascular dementia were similar. The associations between $A\beta_{1-40}$ and $A\beta_{1-42}$ levels and risk of dementia seemed slightly stronger in carriers of an APOE $\epsilon 4$ allele (table 4), however, the interaction terms between APOE genotype and levels of $A\beta_{1-40}$, $A\beta_{1-42}$ and the ratio were far from significant (all $p > 0.5$). The association between plasma $A\beta$ and risk of dementia was similar in persons with and without diabetes mellitus. Because the association with the ratio of $A\beta_{1-40}$ and $A\beta_{1-42}$ was highly significant, we further explored their interrelation by looking at their combined effects in tertiles of both $A\beta_{1-40}$ and $A\beta_{1-42}$. The effect appeared mostly due to a strongly increased risk of dementia in persons with high levels of $A\beta_{1-40}$ combined with low levels of $A\beta_{1-42}$ (age and sex adjusted hazard ratio (95% CI) for dementia for persons with levels in the highest tertile of $A\beta_{1-40}$ combined with levels in the lowest tertile of $A\beta_{1-42}$ compared to persons with levels in the lowest tertiles of both $A\beta_{1-40}$ and $A\beta_{1-42}$ 10.10 (4.29-23.81) (figure 1)).

Table 3. The association between plasma $A\beta$ and subtypes of dementia. Hazard ratio (95% confidence interval)[†] for Alzheimer's disease (289 cases) and vascular dementia (54 cases) per standard deviation (SD) increase in plasma $A\beta$

	$A\beta_{1-40}$	$A\beta_{1-42}$	$A\beta_{1-42} / A\beta_{1-40}$
Alzheimer's disease	1.17 (1.02-1.34)	0.85 (0.63-1.15)	0.55 (0.38-0.80)
Vascular dementia	1.14 (0.89-1.46)	0.82 (0.42-1.62)	0.49 (0.23-1.03)

[†] Adjusted for age and sex

Table 4. The association between plasma $A\beta$ and dementia in carriers (152 incident dementia cases) and non-carriers (231 incident dementia cases) of an APOE $\epsilon 4$ allele. Hazard ratio (95% confidence interval)[†] for dementia per standard deviation (SD) increase in plasma $A\beta$

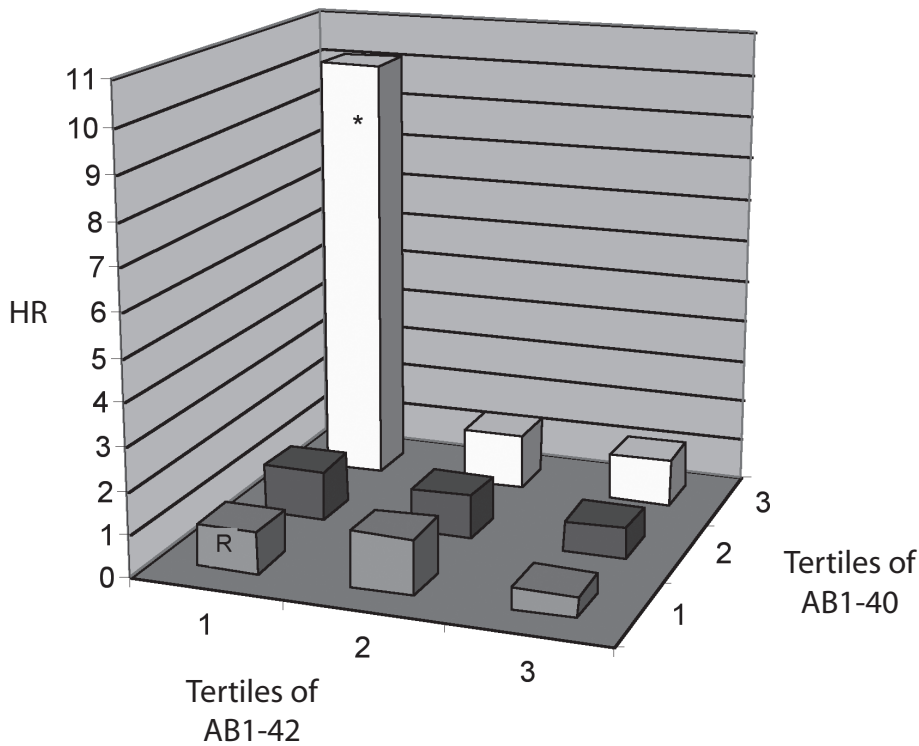
	$A\beta_{1-40}$	$A\beta_{1-42}$	$A\beta_{1-42} / A\beta_{1-40}$
Carriers APOE $\epsilon 4$ allele	1.31 (1.04-1.66)	1.32 (0.69-2.52)	0.72 (0.40-1.29)
Non-carriers APOE $\epsilon 4$ allele	1.14 (0.98-1.32)	0.88 (0.68-1.15)	0.59 (0.39-0.90)
P-value interaction term	0.72	0.63	0.58

[†] Adjusted for age and sex

Figure 1. Hazard ratio (HR) for dementia across different levels of $A\beta_{1-40}$ and $A\beta_{1-42}$

R=reference category (low levels of both of $A\beta_{1-42}$ and $A\beta_{1-40}$)

* $P < 0.05$



Discussion

We found that higher plasma levels of $A\beta_{1-40}$ were associated with an increased risk of dementia, especially for persons who had concomitant low levels of $A\beta_{1-42}$. Persons with higher levels of $A\beta_{1-40}$ combined with low levels of $A\beta_{1-42}$ had an over 10-fold increased risk of dementia compared to persons with low levels of both $A\beta_{1-40}$ and $A\beta_{1-42}$. These associations were independent of the presence of an APOE $\epsilon 4$ allele. Plasma levels of $A\beta_{1-42}$ were not associated with the risk of dementia.

The strengths of the Rotterdam Study include its prospective design, population-based setting, large number of subjects and nearly complete follow-up.



We found similar associations between plasma A β levels and dementia and between plasma A β levels and both major subtypes of dementia, Alzheimer's disease and vascular dementia. This may be related to the fact that most incident patients in our study were diagnosed with Alzheimer's disease. Also, in an elderly population mixed pathology is commonly seen and may explain the similar estimates for Alzheimer's disease and vascular dementia.²¹

Cross-sectional studies that previously examined the relation between plasma A β and dementia yielded inconsistent results.^{10,22-24} Since plasma A β levels are believed to change in the course of the dementia process, longitudinal observations are more useful to study whether A β levels are associated with an increased risk in asymptomatic persons. Thus far only two longitudinal studies have been reported that examined the association between plasma A β and the risk of dementia. In line with our findings, these studies reported associations between plasma A β levels and the risk of dementia. Mayeux et al. found higher baseline plasma A β_{1-40} and A β_{1-42} levels in 86 individuals who developed AD compared with elderly who remained free of dementia over a follow-up period of five years. After adjusting for age, BMI and A β_{1-40} level, only A β_{1-42} levels remained significantly different. Regarding change in plasma A β_{1-40} and A β_{1-42} levels, a significant decline in plasma A β_{1-42} but not plasma A β_{1-40} levels was found in patients with newly acquired AD compared with those with prevalent AD or controls.⁹ More recently, Pomara et al reported that higher baseline plasma A β_{1-42} levels and greater reductions in plasma A β_{1-42} levels were associated with cognitive decline in 34 elderly subjects free of dementia.¹⁴ Both studies found associations of high levels of A β_{1-42} and a decline of A β_{1-42} levels with dementia. These findings seem discrepant with our finding of no association between plasma A β_{1-42} and risk of dementia. We found, however, a strong association with the ratio A $\beta_{1-42}/A\beta_{1-40}$, which implies that lower levels of A β_{1-42} in combination with higher levels of A β_{1-40} , are associated with an increased risk of dementia. Since there is evidence that plasma levels of A β change during the presymptomatic period of dementia, it is possible that differences in timing of the A β measurements with respect to dementia diagnosis are responsible for differences between studies. More prospective studies with long follow-up and repeated measurements of A β are needed to establish the value of plasma A β in identifying persons at risk of with early stage dementia. The question remains what underlies the association between plasma A β levels and risk of dementia. It is generally considered that the brain is the origin of the A β that is deposited in plaques in AD patients.⁹ Since CSF and plasma A β levels are believed to be in a dynamic equilibrium,^{15,25} increased A β production in the brain may be reflected in plasma levels. Although in general plasma A β_{1-40} and plasma A β_{1-42} levels were highly correlated in our study, we found that the risk of dementia was particularly increased in persons with high levels of plasma A β_{1-40} and concomitant low levels of plasma A β_{1-42} . This may reflect the deposition of A β_{1-42} in plaques in the years preceding clinical onset of Alzheimer's disease.¹³ It has been well established that levels of A β_{1-42} decline in CSF of patients with typical late onset AD.²⁶ This may theoretically cause plasma A β_{1-42} to decline because CSF A β , that has a high concentration relative to



plasma A β , is normally cleared in the blood.²⁵ Some studies in APP transgenic mice suggest equilibrium between A β deposited in the brain, soluble A β in CSF, and A β in plasma.¹⁵ Along with the marked deposition of A β ₁₋₄₂ in the brain, there is not only a decline in CSF A β but also a substantial and highly significant decrease in plasma A β ₁₋₄₂. In Alzheimer's disease patients CSF and plasma A β do not clearly correlate.²² However, it should be noted that this correlation might be different in the preymptomatic period of Alzheimer's disease, when there is no or less aggregation of beta amyloid in plaques.

However, alternative explanations are possible. Changes in peripheral clearance of plasma A β may contribute to changes in plasma A β levels. Plasma A β levels are strongly associated with serum creatinine, which indicates that changes in renal function may play a role. Since adjustment for serum creatinine in our analyses did not change the associations, we do not consider this a likely explanation for our findings. A β is also produced peripherally in the blood. The association of plasma levels with risk of dementia would be compatible with peripheral sources of A β being responsible for A β brain deposits. Although this has been suggested,²⁷ generally the brain is believed the primary source.⁹

A different explanation for our findings may follow from the notion that plasma A β , especially A β ₁₋₄₀, can be causally involved in microvascular dysfunction.²⁸ Two recent cross-sectional studies show an association between plasma A β and cerebral small vessel disease.^{8,29} In the Rotterdam Study, we found that both plasma A β ₁₋₄₀ and A β ₁₋₄₂ levels were associated with more lacunar infarcts and white matter lesions in persons who carried an APOE ϵ 4 allele.⁸ More recently, Gurol et al. showed that plasma A β ₁₋₄₀ was independently associated, regardless of APOE ϵ 4 carrier status, with extent of white matter hyper intensity in persons with AD, mild cognitive impairment, or cerebral amyloid angiopathy.²⁹ Given that small vessel disease is related to risk of Alzheimer's disease,³⁰ the link between plasma A β levels and Alzheimer's disease may reflect microvascular involvement in the pathogenesis of Alzheimer's disease. It is, however, also conceivable that the association is explained the other way round; ischemic microvascular disease may promote release of brain A β into the circulation.²⁹

It should be noted that the source of plasma A β is not known. APP is produced by a variety of cells, including cells in and outside the brain, such as platelets.

Though it remains unclear whether the dementia disease process in the brain affects or is affected by plasma A β , this study provides evidence that plasma A β levels are associated with risk of dementia. A potential role of plasma A β levels as a marker of incipient dementia warrants further investigation.



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Chapter 5. General discussion

The main objective of the research described in this thesis was to gain more insight in inflammatory and vascular mechanisms involved in the pathogenesis of dementia. A secondary aim was to identify preclinical markers that may be useful for early detection of dementia. All studies were performed within the framework of the Rotterdam Study, a large prospective population-based cohort study among persons of 55 years and older.¹ In the following paragraphs I will briefly review our main findings and discuss how they relate to current knowledge regarding the development and early detection of dementia. Finally, I will consider the clinical implications of our findings and comment on future research topics.

5.1 Main findings in the light of current knowledge

In this paragraph I will review our main findings in the light of current knowledge and discuss various mechanisms that may underlie our findings.

5.1.1 *Inflammation and dementia*

Many studies indicate that an inflammatory process in the brain plays a role in the development of dementia. Signs of inflammation such as activated microglia and inflammatory mediators, including C-reactive protein and complement factors, are present in the brain of demented persons and it is generally thought that this inflammatory process contributes to neuronal death.²

The role of peripheral inflammation, however, is less clear. Several studies reported increased serum levels of pro-inflammatory cytokines in patients with Alzheimer's disease.^{3,4} Few prospective studies showed an association between inflammatory proteins in plasma including C-reactive protein and Alpha1-antichymotrypsin (ACT), and the risk of dementia,^{5,6} suggesting that these proteins are elevated before the onset of dementia and therefore may be involved in the development of dementia.

In **chapters 2.1** and **2.2** I described the associations that we found between plasma levels of two peripheral markers of inflammation and the risk of dementia. Higher plasma levels of Lp-PLA2 and fibrinogen were associated with an increased risk of dementia, both Alzheimer's disease and vascular dementia. We could not confirm an association between higher plasma levels of C-reactive protein and an increased risk of dementia (**chapter 2.2**). Besides plasma levels of inflammatory proteins, variation in genes that influence levels of inflammatory proteins may provide additional evidence for a causal role of these proteins in the pathogenesis of dementia. In **chapter 2.3** we observed an association between common variation in the C-reactive protein gene and the risk of dementia. The variant that was related to higher plasma levels of C-reactive protein was associated with an increased risk of dementia, particularly in carriers of the APOE $\epsilon 4$ allele. These findings seem contradictory to





our finding that plasma levels of C-reactive protein were not associated with risk of dementia. However, plasma levels measured in late-life may not well reflect cumulative C-reactive protein exposure and since genetic determinants of C-reactive protein underlie lifelong exposure level, this might explain our different results with levels and the genetic variation. No evidence was found for a causal relation of interleukin 6 and transforming growth factor β 1 with dementia when we examined genetic variation in genes that influence plasma levels of these inflammatory proteins (**chapter 2.5**).

We propose several mechanisms through which inflammatory markers may be linked to an increased risk of dementia. Since Lp-PLA2, fibrinogen and C-reactive protein have, consistently and independently of traditional cardiovascular risk factors, been associated with risk of cardiovascular disease,^{7,8} a vascular pathway may explain the association with dementia. Perhaps, peripheral markers of inflammation reflect the important inflammatory components of the process of atherosclerosis.⁹ There is clear evidence that atherosclerosis is associated with an increased risk of dementia.¹⁰ Peripheral markers of inflammation may also reflect cerebral small vessel disease, indicated by silent brain infarcts and white matter lesions. Both silent brain infarcts and white matter lesions may lead to dementia.^{11,12} Associations of both C-reactive protein and fibrinogen with small vessel disease have been observed,^{13,14} though we could not replicate the latter association in the Rotterdam Scan Study (**chapter 2.4**).

Instead of merely reflecting vascular disease, causal roles for Lp-PLA2, fibrinogen, and C-reactive protein, in atherogenesis, plaque formation and hemostasis have been suggested.¹⁵⁻¹⁷ In our studies the associations between peripheral inflammatory markers and dementia were independent of vascular risk factors, including measures of atherosclerosis. However, vascular factors we controlled for may not have adequately reflected (cerebro) vascular disease. Considering the existing evidence, a vascular mechanism, either reflected or caused by peripheral inflammatory markers, seems a plausible explanation for our findings. Also the finding of stronger associations of both Lp-PLA2 and fibrinogen levels with vascular dementia lends support to a vascular pathway.

Elevated levels of peripheral inflammatory markers may be a consequence of the dementia disease process. It has been suggested that C-reactive protein produced in the course of the local inflammatory process in the brain may pass the blood-brain barrier and appear in the periphery.¹⁸ Alternatively, inflammatory cytokines produced in this process may trigger peripheral production of inflammatory proteins.¹⁹

An alternative explanation for our findings is that peripheral inflammation is directly related to dementia i.e. not through a vascular mechanism. Minimal evidence exists for a direct causal role of inflammatory proteins in the pathogenesis of dementia. ACT, for instance, is believed to reinforce the formation of beta-amyloid deposits²⁰ but whether C-reactive protein, fibrinogen or Lp-PLA2 exert direct effects on (formation of) Alzheimer pathology is not known.





5.1.2 Vascular factors and dementia

Over the last decade, evidence for a role of vascular factors in the development of dementia, both Alzheimer's disease and vascular dementia, has been accumulating.^{21,22} In his first case report in 1907 Alois Alzheimer already reported microvascular changes, in addition to plaques and tangles. Histopathological studies showed a high prevalence of cerebrovascular lesions in Alzheimer patients.⁶ In addition, 30 to 50% of persons with a diagnosis of vascular dementia have histological lesions of Alzheimer's disease,²³ indicating major overlap between vascular and Alzheimer's pathology.

We reported an association of carotid atherosclerosis, high blood pressure and myocardial infarction with dementia, both Alzheimer's disease and vascular dementia, independent of other vascular risk factors. Arterial stiffness, measured as pulse wave velocity and carotid distensibility, was not found to be an independent predictor of cognitive decline or dementia (**chapter 3.3**).

In line with a previous report from the Rotterdam Study¹⁰ we found that indicators of atherosclerosis were associated with an increased risk of dementia (**chapter 3.1**).

Our finding of an increased risk of dementia associated with higher systolic and diastolic blood pressure in persons younger than 75 years (**chapter 3.2**) is in accord with the view that hypertension, especially in midlife, negatively affects cognition and contributes to the development of dementia later in life.²⁴ There is evidence that low blood pressure later in life may exert harmful effects on cognition through hypoperfusion of the cerebral circulation,²⁵ which may explain our finding of an association between decline in blood pressure and increased risk of dementia (**chapter 3.2**). Alternatively, decline in blood pressure might be a consequence of the dementia process, since several areas that are involved in the central blood pressure regulation are affected in Alzheimer's disease.²⁶

In **chapter 3.4** we showed that myocardial infarction was associated with risk of dementia, which is in agreement with a recent report that showed an association between coronary artery disease and Alzheimer neuropathology.²⁷ Unrecognized, but not recognized, myocardial infarction was associated with a more than 2-fold increased risk of dementia in men, but not in women. An explanation why unrecognized but not recognized myocardial infarction was associated with risk of dementia may be that persons with recognized MI are more often treated with cardiovascular drugs and more often quit smoking and change their diet. The difference in risk between the sexes is in line with findings from other studies where unrecognized MI carried a higher risk for cardiovascular death and stroke in men but it is not clear what explains this difference.

How can we link vascular disease and vascular risk factors to dementia? First, cerebrovascular disease may mediate the associations with dementia. Indicators of atherosclerosis, hypertension and myocardial infarction are all established risk factors for stroke. However,





the associations with dementia remained when we accounted for clinical stroke in the analyses, indicating that another mechanism must play a role. Indicators of atherosclerosis, hypertension and myocardial infarction have also been associated with cerebral small vessel disease,^{28,29} reflected by lacunar infarcts and white matter lesions, which may explain the relation with an increased risk of dementia.^{11,12} Because imaging of the brain was not routinely performed in all participants we were not able to assess this in our studies. Second, the concept of cerebral hypoperfusion may partially explain our findings. Hypoperfusion of the cerebral vasculature may destabilize neurons and synapses, and evolve into a neurodegenerative process characterized by formation of senile plaques, neurofibrillary tangles and amyloid angiopathy.²⁴

Finally, the possibility that the associations are not causal cannot be completely ruled out. Vascular disease and dementia may be independent processes with common elements in the causal pathway.³⁰ However, the prospective nature of our study, and the fact that the associations remained after adjustment for many potential confounders, supports a causal association between vascular factors and dementia.

Most likely, vascular risk factors and mechanisms proposed to explain our findings are interrelated and interact in their association with dementia. For example, long-term high blood pressure can cause severe atherosclerosis, which in turn may lead to dementia.

5.1.3 Markers for early detection of dementia

Subjective memory complaints and dementia

That subjective memory complaints predict risk of dementia is plausible and in line with other population-based studies.^{31,32} Our study is the first to show that memory complaints are most predictive of Alzheimer's disease in persons with a high level of education that perform well on the cognitive screening test (MMSE) (**chapter 4.1**). We also demonstrated, in line with previous studies,^{33,34} that persons with a high level of education had a decreased risk of dementia. This latter observation may be explained by the hypothesis that education provides "cognitive reserve"³⁵ but an equally plausible explanation may be that Alzheimer's disease in highly educated persons is simply under-detected.

Our finding of a strong association between subjective memory complaints and Alzheimer's disease especially in highly educated persons without objective cognitive impairment is suggestive of an under-detection of early stage dementia in these persons. Likely, these persons experience cognitive deficits related to early stage dementia, not detected by formal cognitive screening tests, and therefore complain about their memory. Our data indicate that in highly educated persons subjective memory complaints should not be discarded when screening tests do not show cognitive deficits. Though we were not able to examine this in our study, in these cases more extensive neuropsychological testing may be indicated to detect dementia.





Plasma A β and dementia

Though it is unclear how plasma A β concentrations relate to A β metabolism in the brain, there is evidence that plasma A β concentrations are associated with risk of dementia.³⁶ We showed that high plasma concentrations of A β_{1-40} were associated with an increased risk of dementia, especially in persons who have concomitant low concentrations of A β_{1-42} (**chapter 4.2**) which is in accord with preliminary findings of Graff-Radford and colleagues.³⁷ Individuals with high concentrations of A β_{1-40} combined with low concentrations of A β_{1-42} had an over 10-fold increased risk of dementia compared with people with low concentrations of both A β_{1-40} and A β_{1-42} (**chapter 4.2**).

Several mechanisms may underlie this association. Since CSF and plasma A β concentrations are believed to be in a dynamic equilibrium,³⁸ increased A β production in the brain may be reflected in raised plasma concentrations of A β . Similarly, deposition of A β_{1-42} in plaques in the years preceding clinical Alzheimer's disease may be reflected in low plasma concentrations of A β_{1-42} .³⁷ Indeed, studies in APP transgenic mice show that along with deposition of A β_{1-42} in the brain, there is not only a decline in CSF A β , but also a highly significant decrease in plasma A β_{1-42} .³⁸ Alternative explanations are that plasma A β is associated with dementia through microvascular dysfunction. Several studies have shown an association between plasma A β and lacunar infarcts and white matter lesions,^{39,40} that may mediate the association with dementia.¹¹ The other way round, ischaemic microvascular disease might promote release of brain A β into the circulation.³⁹

Though the value of plasma A β in identifying individuals at risk of dementia still needs to be established, our results provide evidence that plasma A β concentrations are associated with risk of dementia and may be used as a marker of incipient dementia.

5.2. Methodological considerations

Specific methodological issues have been discussed in the relevant chapters. In the following paragraph I will address more general methodological considerations related to the design of the Rotterdam Study, difficulties in detecting and diagnosing dementia and subtypes of dementia, genetic association studies and studies with a long follow-up period.

Study design

Several methodological considerations regarding the design of the Rotterdam Study, in which all studies were embedded, need to be discussed. First, the prospective nature of the study enabled us to collect data on exposure variables (inflammatory, vascular factors and preclinical makers) before persons became clinically demented, thus making causal inference possible. However, since the subclinical course of dementia and Alzheimer's disease is believed to take many years, it is possible that despite the long follow-up period in our studies, characteristics measured at baseline were consequences of the disease process. This would mean that





associations of these characteristics with risk of dementia may not be explained by a causal mechanism but merely reflect the early dementia process. Second, selective loss to follow-up may have biased our results. To ensure minimal loss to follow-up regarding dementia, we continuously monitored for incident dementia through computerized linkage between the study database and medical records from general practitioners and the Regional Institute for Outpatient Mental Healthcare (RIAGG) but since early stages of dementia are not always recognized or reported in the medical records, dementia cases may have been missed.

Detection and diagnosis of dementia

In order to detect signs of dementia we used a three-step protocol. The first step of screening involved two brief cognitive screening tests (the mini mental state examination (MMSE) and the Geriatric Mental State Schedule (GMS)). Because both tests are not sensitive and performance on these strongly depends on level of education, we may have missed early dementia cases, particularly in persons with a higher education.

Regarding diagnosis of dementia the following considerations need to be taken into account. The combination of symptoms that we consider the syndrome of dementia should be regarded as the end-stage of cognitive decline that has been ongoing for many years. Naturally, this process is a continuum and there is not one particular moment in time when a person becomes demented. Hence, dementia as defined by the current diagnostic criteria, should be regarded as the end of the cognitive decline-spectrum. Diagnostic criteria for the syndrome of dementia have been subject to changes over the years; also indicating this diagnosis is arbitrary. Since no test exists that indisputably proves dementia, cognitive test results, together with the patient's history, an informant interview, and preferably brain imaging, are used to establish the diagnosis. For reasons of consistency, we used criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM III-R) for the diagnosis of dementia, NINCDS-ADRDA⁴¹ criteria for the diagnosis of Alzheimer's disease and NINDS-AIREN⁴² criteria for diagnosis of vascular dementia, that are widely used and accepted in both clinical practice and research settings.

Furthermore, distinction between different subtypes of dementia, especially between Alzheimer's disease and vascular dementia, is questionable. Since studies show considerable overlap in risk factors,²¹ neuropathology,⁴³ and clinical features of both subtypes, the usefulness of this distinction is minimal. At best, one may consider vascular dementia as the dementia subtype where involvement of a vascular mechanism is most likely. To be consistent with other studies, we made the distinction between subtypes in subanalyses but we focused on total dementia as the primary outcome.





Genetic association studies

In genetic association studies some sources of bias can be more easily avoided than in classic epidemiological studies.⁴⁴ For example, reversed causality, which can be a major problem in classic epidemiological studies, is not an issue. Still, some methodological issues specific to these studies need to be addressed. The main problem in genetic association studies is the lack of power to find an association. This is especially true when studying a common complex disorder (like sporadic Alzheimer's disease) that is believed to result from both genetic and environmental factors that may interact. Since the effect of an individual polymorphism on the development of dementia is likely to be small, it may not be detected. In our studies, this problem may have been overcome because of the large study population and the large number of dementia cases. Also, in general the use of haplotypes, that describe the total common variation of a gene, increases power to detect associations.⁴⁵ Another advantage of haplotype-analysis is that it decreases the risk of false positive results, which may be an issue when multiple genetic polymorphisms are studied.

Long follow-up studies

In general, studies with a long period of observation are preferred because they can best establish whether associations are causal. However, some considerations regarding these long follow-up studies need to be addressed.

When investigating vascular factors (indicators of atherosclerosis (**chapter 3.1**) and blood pressure (**chapter 3.2**)) in relation to risk of dementia, we observed attenuation of the associations when we examined cases that occurred during longer follow-up. This may have been due to strong associations of these vascular factors with risk of mortality.

Additionally, loss to follow-up is an increasing problem over a longer period of follow-up and as the age of the surviving cohort increases, diagnosing dementia will be more difficult, and possibly less a priority, because of comorbidity. These problems may result in an underestimation of the number of late dementia cases and may bias the results, possibly making it difficult to find associations during longer follow-up.

5.3 Clinical implications

The notion that inflammatory and vascular factors play a causal role in the development of dementia may lead to new treatment or prevention strategies.

Our studies suggest that treatment of vascular disease and risk factors, such as atherosclerosis and high blood pressure, might reduce dementia incidence. Currently, evidence for this is still largely lacking. The influence of anti-hypertensive use on the incidence of dementia has been most extensively studied and several randomized placebo-controlled trials showed a lower incidence of dementia associated with anti-hypertensive use.^{46,47} While issues such as the optimal timing of treatment and choice of anti-hypertensive drug, still need to be





resolved these results seem promising. It should be noted, however, that no convincing evidence exists for beneficial effects of treatment of vascular factors on cognition in dementia patients. Therefore, prevention of dementia should be the focus. Though more evidence on efficacy is needed, it is conceivable that treatment of vascular risk factors may reduce the risk of dementia. Also, life style changes directed at modifying vascular risk factors, such as changing dietary patterns and smoking habits, and engaging in physical exercise, may also have beneficial effects in development of dementia. Indeed, physical activity has been associated with a reduced risk of dementia and Alzheimer's disease. Screening programs designed to detect and treat cardiovascular risk factors, such as high cholesterol, hypertension and, possibly, unrecognized myocardial infarction, in order to prevent cardiovascular disease may also prevent dementia.

For early intervention preclinical markers to identify dementia patients in an early stage or persons at high risk of dementia are needed. Our finding that subjective memory complaints were predictive of dementia before cognitive deficits could be detected by screening tests in highly educated persons may be useful to identify persons at high risk of dementia in clinical practice. We also demonstrated an association of plasma A β levels with risk of dementia. However, replication of these findings is needed and the value of plasma A β levels in identifying persons at risk for dementia in clinical practice needs to be established.

5.4 Future research topics

Research should focus on elucidating mechanisms through which vascular factors and vascular disease may cause dementia. One proposed mechanism is cerebral hypoperfusion. Hypoperfusion of the cerebral vasculature may destabilize neurons and synapses, and evolve into a neurodegenerative process.²⁴ Cerebral hypoperfusion as a causal pathway for development of dementia warrants further investigation. In this respect, examining the association between cardiac disease, such as heart failure,⁴⁸ and dementia, and replication of our finding of a relation between (unrecognized) myocardial infarction and dementia seems worthwhile.

To evaluate the role of endothelial damage of blood vessels in the brain that is related to cerebral hypoperfusion,²² the association between markers of endothelial damage, such as von Willebrand Factor (vWF), and dementia may be of interest. We showed an effect of plasma levels of fibrinogen on the risk of dementia. Fibrinogen may be associated with dementia through affecting endothelial function and reducing blood flow.¹⁶ To further establish the potential causal role of plasma fibrinogen it is of interest to study haplotypes in the fibrinogen β (FGB) gene that affect plasma levels of fibrinogen. Additionally, examining the influence of other components of the coagulation cascade on the risk of dementia may provide new insights in the role of hemostasis in the pathogenesis of dementia.

An important issue remains establishing causality of vascular factors in the association with





dementia. Since the possibility exists that vascular disease simply co-exists with presence of dementia, a stronger case should be made out for causality of these associations. The ultimate tool for establishing causality of vascular factors in dementia is a randomized placebo controlled trial. However, denying persons with established vascular disease or risk factors treatment will not be possible on ethical grounds. Other difficulties involved are determining the proper study population and timing of the intervention, which should ideally be before the onset of dementia.

Hence, other methods of establishing causal associations are needed. Whether hypoperfusion precedes the dementia process, a prerequisite for causation, can be examined by measuring cerebral blood flow before onset of disease. This can be done non-invasively and in large-scale population-based studies by means of MRI techniques. Prospectively examining midlife inflammatory and vascular factors in a young population in relation to incidence of dementia at old age will provide stronger evidence for a causal relation. To avoid survival bias, complete information on morbidity, especially incidence of dementia, and mortality will be essential. Recently, the Rotterdam Study started a younger cohort (aged 45 years and over) in which studies like these can be performed. Another promising approach to establish causality of inflammatory and vascular factors in the association with dementia is to study genetic variation. Variation in inflammatory genes or genes related to cardiovascular factors can be examined in relation to incidence of dementia. In this respect the genomic wide screen that is planned for all Rotterdam Study participants will yield a huge amount of information. To minimize false positive findings and increase power of detecting small effects haplotype-analyses are preferred and recently software packages have become available that can adequately perform these analyses in large studies.

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Chapter 6. Summary/Samenvatting

Summary

Though dementia, particularly Alzheimer's disease, is a very common disease in the elderly, the precise etiology is not known. Several mechanisms that may play a role in the development of dementia have been proposed, including inflammatory and vascular mechanisms. However, it is unclear whether these mechanisms actually cause dementia. Possibly, they simply reflect the process of dementia or are related to independent co-existent disorders.

The main objective of the research in this thesis was to examine the potentially causal role of inflammatory and vascular factors in the development of dementia. Subsequently, we sought to identify preclinical markers that may be of use in early detection of dementia.

All research was performed within the Rotterdam Study, a large prospective population-based cohort study among 7,983 persons of 55 years and over with assessment of inflammatory and vascular factors at baseline. Patients with dementia were identified by means of a multi-step cognitive screening protocol at the research center and linkage of the study database with medical records of the general practitioner and the outpatient mental health care.

Chapter 2 dealt with the relation between inflammatory factors and risk of dementia. First, we examined the influence of markers of inflammation in plasma on the risk of dementia and observed that higher plasma levels of Lp-PLA2 (**chapter 2.1**) and fibrinogen (**chapter 2.2**) were associated with an increased risk of dementia. These associations were independent of (other) inflammatory or vascular factors, not explained by occurrence of clinical stroke, and strongest for vascular dementia. These findings suggested that Lp-PLA2 and fibrinogen might play a role in the pathogenesis of dementia. Second, we examined variation in inflammatory protein genes in relation to dementia risk. In **chapter 2.3** we found that the variant of the C-reactive protein gene that was related to higher plasma levels of C-reactive protein was associated with an increased risk of dementia, indicating a causal role for C-reactive protein in the development of dementia. In **chapter 2.4** we evaluated plasma fibrinogen levels and variation in the fibrinogen β (FGA) and fibrinogen β (FGG) genes, believed to be involved in regulating fibrin clot structure, in relation to cerebral small vessel disease (SVD) as reflected by silent brain infarcts and white matter lesions on brain imaging. We found that common variation in the FGA and FGG genes was associated with cerebral SVD, whereas fibrinogen levels were not, suggesting that structure of the fibrin clot may play a role in the pathogenesis of SVD rather than plasma fibrinogen levels. No association was found of common variation in the FGA and FGG genes with dementia (**chapter 2.5**). Polymorphisms in the interleukin 6 (IL-6) gene, related to plasma levels of IL-6, and the transforming growth factor β 1 (TGF β 1)





gene, related to plasma levels of TGF β 1, were not associated with risk of dementia (**chapter 2.6**).

In **chapter 3** vascular factors were examined in relation to dementia. We found that indicators of atherosclerosis, predominantly carotid atherosclerosis, were associated with an increased risk of dementia (**chapter 3.1**). However, the associations were mainly confined to cases that occurred during short follow-up. We also found a strong association between all indicators of atherosclerosis and risk of mortality. Our findings support the hypothesis that atherosclerosis is involved in the pathogenesis of dementia and Alzheimer's disease. Associations attenuated with increasing duration of follow-up, probably due to selective mortality in persons with severe levels of atherosclerosis. Higher levels of systolic and diastolic blood pressure were associated with an increased risk of dementia (**chapter 3.2**). This increased risk was restricted to persons younger than 75 years. A decline in both systolic and diastolic blood pressure, defined as a decrease between baseline and the first follow-up measurement of ≥ 5 mm Hg, was associated with increased risk of dementia. This study supported the hypothesis that high blood pressure, especially in younger persons, is associated with an increased risk of dementia. In **chapter 3.3** we found an association between arterial stiffness (measured by assessment of pulse wave velocity (PWV) and carotid distensibility (CD)) and several domains of cognitive function. However, after adjustment for cardiovascular risk factors, only PWV was associated with poor performance on the Stroop. Since no independent associations were found between arterial stiffness and cognitive decline or risk of dementia, we concluded that arterial stiffness is not an independent predictor of cognitive decline or risk of dementia. Unrecognized, but not recognized, myocardial infarction was associated with a more than 2-fold increased risk of dementia in men, but not in women (**chapter 3.4**). An explanation why unrecognized but not recognized myocardial infarction was associated with risk of dementia may be that persons with recognized MI are more often treated with cardiovascular drugs and more often quit smoking and change their diet. The difference in risk between sexes is in line with findings from other studies where unrecognized MI carried a higher risk for cardiovascular death and stroke in men but it is not clear what explains this difference. Our findings suggested that coronary heart disease is associated with an increased risk of dementia.

In **chapter 4** we presented markers for early detection of dementia. Subjective memory complaints predicted dementia, particularly in higher educated persons who performed well on the baseline mini mental state examination (MMSE) (**chapter 4.1**). Our data indicated that in highly educated persons subjective memory complaints should not be discarded when screening tests do not show cognitive deficits. Though we were not able to examine this in our study, in these cases more extensive neuropsychological testing may be indicated to detect dementia. In **chapter 4.2** we examined the association between plasma $A\beta$ concentrations and risk of dementia. We showed that high concentrations of plasma $A\beta_{1-40}$ especially when combined with low concentrations of plasma $A\beta_{1-42}$ were associated with an increased risk of





dementia. Though the value of plasma A β in identifying individuals at risk of dementia still needs to be established, our results provided evidence that plasma A β concentrations may be used as a marker of incipient dementia.

In **chapter 5** I reviewed our main findings in the light of current knowledge. Several mechanisms were discussed that may link inflammation and vascular factors to dementia. Furthermore, methodological considerations regarding our studies, clinical implications and future research topics were brought up.

Samenvatting

Dementie, met name de ziekte van Alzheimer, is een veel voorkomende aandoening bij ouderen. Echter, tot op heden is het niet duidelijk wat de oorzaak is van het dementiesyndroom. Verschillende mechanismen, zoals ontstekingsgerelateerde mechanismen en vasculaire mechanismen, zouden een rol kunnen spelen bij het ontstaan van dementie. Het is echter de vraag of deze mechanismen daadwerkelijk dementie veroorzaken of dat ze een reflectie zijn van het dementie-proces of onafhankelijk bestaan naast dementie.

De belangrijkste doelstelling van de studies beschreven in dit proefschrift was te onderzoeken of er een oorzakelijk verband bestond tussen verschillende ontstekingsgerelateerde en vasculaire factoren en de kans op dementie. Daarnaast zochten we naar preklinische markers die zouden kunnen helpen dementie in een vroeger stadium te diagnosticeren.

Alle studies maakten deel uit van het Erasmus Rotterdam Gezondheid en Ouderen (ERGO) onderzoek, een groot prospectief bevolkingsonderzoek onder 7983 personen van 55 jaar en ouder. Bij aanvang van de studie werden mensen onderzocht en gegevens verzameld met betrekking tot ontstekingsgerelateerde en vasculaire (risico)factoren. Alle deelnemers werden tijdens diverse onderzoeksrondes onderzocht op tekenen van dementie. Daarnaast hadden we toegang tot de medische dossiers van de huisarts en het RIAGG. Op deze manier konden we voor iedereen vaststellen of er dementie was opgetreden tijdens de onderzoeksperiode.

Hoofdstuk 2 behandelde de relatie tussen ontstekingsgerelateerde factoren en de kans op dementie. Eerst onderzochten we de invloed van ontstekingsmarkers in plasma op het dementierisico. We zagen dat hogere concentraties Lp-PLA2 (**hoofdstuk 2.1**) en fibrinogeen (**hoofdstuk 2.2**) waren geassocieerd met een verhoogde kans op dementie. Deze associaties waren onafhankelijk van (andere) ontstekingsgerelateerde en vasculaire factoren, onafhankelijk van het optreden van een beroerte en het sterkst voor vasculaire dementie. Daarnaast onderzochten we of variatie in genen die coderen voor ontstekingseiwitten invloed had op de kans op dementie. In **hoofdstuk 2.3** vonden we dat de variant van het CRP gen die zorgt voor hogere CRP plasma concentraties een verhoogde kans op dementie gaf, hetgeen wees op een oorzakelijke rol voor CRP in het ontstaan van dementie. In **hoofdstuk 2.4** beschreven we de invloed van fibrinogeen plasma concentraties en variatie in fibrinogeen β (FGA) en fibrinogeen β (FGG) genen, van belang voor regulatie van de stolselstructuur,





op het voorkomen van cerebrale microangiopathie. We vonden dat variatie in de FGA en FGGen was geassocieerd met meer cerebrale microangiopathie op de hersenscan. Omdat we geen aanwijzingen vonden voor een associatie tussen plasma fibrinogeen en cerebrale microangiopathie, concludeerden we dat de structuur van het stolsel mogelijk belangrijker was in de relatie met cerebrale microangiopathie dan de plasma fibrinogeen concentratie. We vonden geen relatie tussen variatie in de FGA en FGGen en dementie (**hoofdstuk 2.5**). Polymorfismen in het interleukine 6 (IL-6) gen, gerelateerd aan hogere plasma IL-6 concentraties, en het transforming growth factor $\beta 1$ (TGF $\beta 1$) gen, gerelateerd aan hogere plasma TGF $\beta 1$ concentraties, waren niet geassocieerd met de kans op dementie (**hoofdstuk 2.6**).

In **hoofdstuk 3** beschreven we verschillende vasculaire factoren in relatie tot dementie. We vonden dat atherosclerose, met name in de carotiden, een verhoogde kans op dementie gaf (**hoofdstuk 3.1**). Echter, dit gold hoofdzakelijk voor dementie gedurende de eerste jaren na het begin van de studie. Daarnaast vonden we dat mensen met meer atherosclerose een verhoogd sterfterisico hadden. Onze bevindingen sloten aan bij de hypothese die stelt dat atherosclerose een rol speelt in de ontwikkeling van dementie. Associaties met dementie namen af bij toenemende follow-up duur, mogelijk door selectieve sterfte van personen met ernstige atherosclerose. Hoge systolische en hoge diastolische bloeddruk waren geassocieerd met een verhoogde kans op dementie (**hoofdstuk 3.2**). De verhoogde kans beperkte zich tot personen jonger dan 75 jaar. Een afname in systolische en diastolische bloeddruk, gedefinieerd als een daling tussen de baseline en de eerste follow-up meting van ≥ 5 mm Hg, was gerelateerd aan een verhoogde kans op dementie. Deze studie bevestigde dat hoge bloeddruk, met name in jongere personen, een rol speelt in de ontwikkeling van dementie. In **hoofdstuk 3.3** beschreven we de relatie tussen arteriële stijfheid (gemeten als pulse wave velocity (PWV) en carotid distensibility (CD)) met cognitie en dementie. We vonden dat personen met arteriële stijfheid vaker slecht presteerden op verschillende cognitieve testen. Echter, rekening houdend met andere cardiovasculaire factoren, vonden we slechts een associatie tussen arteriële stijfheid en slechte prestatie op de Stroop test. Omdat we geen onafhankelijke associaties konden vaststellen tussen arteriële stijfheid en cognitieve achteruitgang en een verhoogde kans op dementie, beschouwden we arteriële stijfheid niet als een belangrijke predictor voor cognitieve achteruitgang of dementie. Stille, maar niet klinisch herkende, hartinfarcten gaven een twee maal hogere kans op dementie bij mannen (**hoofdstuk 3.4**). Een mogelijke verklaring voor het feit dat alleen stille infarcten geassocieerd waren met een verhoogde kans is dat mannen met een klinisch herkend infarct vaker behandeld werden met medicijnen tegen hart-en vaatziekten, vaker stopten met roken en bijvoorbeeld vaker hun eetgewoonten aanpasten. Het verschil tussen mannen en vrouwen werd ook gezien in andere studies die vonden dat een stil hartinfarct met name in mannen een verhoogde kans gaf op bijvoorbeeld een beroerte en cardiovasculaire mortaliteit. Het is vooralsnog onduidelijk wat





ten grondslag ligt aan dit sekseverschil. Onze bevindingen wezen op associatie tussen coronair ziekten en een verhoogde kans op dementie.

In **hoofdstuk 4** behandelden we markers voor vroege opsporing van dementie. Subjectieve geheugenklachten voorspelden dementie, voornamelijk in hoger opgeleiden die aan het begin van de studie goed presteerden op een korte cognitieve screeningstest (MMSE)(**hoofdstuk 4.1**). We stelden dat subjectieve geheugenklachten bij hoger opgeleiden met een goede score op een screeningstest serieus genomen moeten worden. Hoewel we dit niet in onze studie konden onderzoeken, zou uitgebreider neuropsychologisch onderzoek mogelijk geïndiceerd zijn om vroege dementie op te sporen bij hoger opgeleiden met subjectieve geheugenklachten. In **hoofdstuk 4.2** bestudeerden we de relatie tussen plasma $A\beta$ concentraties en de kans op dementie. We toonden aan dat een hogere concentratie $A\beta_{1-40}$, met name in combinatie met een lagere concentratie $A\beta_{1-42}$ geassocieerd was met een verhoogde kans op dementie. Mogelijk zou de concentratie van $A\beta$ in plasma gebruikt kunnen worden als marker voor beginnende dementie.

In **hoofdstuk 5** plaatste ik onze bevindingen in het perspectief van de huidige inzichten met betrekking tot het ontstaan van dementie. Verschillende mechanismen die ontsteking en vasculaire factoren aan dementie verbinden werden bediscussieerd. Daarnaast besprak ik de relevantie van onze onderzoeksresultaten voor de klinische praktijk en toekomstig onderzoek.



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About the Author

Marieke van Oijen was born on June 19th, 1977 in Arnhem, the Netherlands. She graduated in 1995 at “Gymnasium Beekvliet” in St. Michielsgestel and started her medical study at the University of Utrecht in the same year. During this period she participated in an elective in emergency medicine for three months in Belo Horizonte, Brazil, and in a research project on herpes simplex encephalitis at the neuro-immunology laboratory of the Minneapolis Medical Research Foundation in Minneapolis, Minnesota, for six months. She also participated in a research project on epilepsy surgery in childhood at the Department of Pediatric Neurology (Prof.dr. O. van Nieuwenhuizen) at the Wilhelmina Children’s Hospital in Utrecht. She graduated from medical school in 2002. After working as a Psychiatry resident, she started the work described in this thesis in 2003 in the Neuroepidemiology group of the Department of Epidemiology & Biostatistics (Prof.dr. M.M.B. Breteler) in collaboration with the Department of Neurology (Prof.dr. P.J. Koudstaal) of the Erasmus Medical Center in Rotterdam. In 2005 she obtained a Master of Science in Clinical Epidemiology at the Netherlands Institute for Health Sciences in Rotterdam. In 2006, she visited Boston to participate in the Nurses’ Health Study of the Harvard School of Public Health and Harvard Medical School for three months. In February 2007 she plans to work at a leprosy clinic in Bauru, Brazil, before starting her Neurology residency at the Erasmus Medical Center (Prof. dr. P.A.E. Sillevs Smitt) on July 1st, 2007.







