

Biomarkers and Risk Factors of Dementia

Elisabeth Marije Caroline Schrijvers

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Biomarkers en risicofactoren van dementie

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Prof. dr. P.J. Koudstaal

Overige leden: Prof. dr. H. Boersma
Prof. dr. C. Brayne
Prof. dr. P. Scheltens

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Introduction

Dementia is a devastating disease that is common in elderly people. The prevalence increases from almost 1% at age 65 to over 40% of people older than 90 years.¹ Because the population is aging, the number of people living with dementia worldwide is expected to double every 20 years with an expected number of 81 million people with dementia in 2040.² Currently, dementia is still a clinical diagnosis of disturbances in cognitive functions that interfere with normal daily functioning.^{3,4} The major subtype of dementia is Alzheimer disease, which based on a clinical diagnosis, accounts for around 70% of all dementia. The second most common subtype is vascular dementia, which is diagnosed in about 15% of dementia cases.¹ Nowadays, however, we know that most patients with dementia have a mix of the neurodegenerative changes that typically occur in Alzheimer disease, vascular pathology and other pathological signs, like Lewy bodies.^{5,6} In addition, vascular risk factors have repeatedly been associated with not only vascular dementia, but also with Alzheimer disease,⁷ supporting the neuropathological findings of mixed brain pathologies. Although many risk factors for dementia have been identified in the past decades, the exact mechanisms that lead to dementia are still unclear. When the clinical diagnosis of dementia is made, the actual neuropathological processes that have led to dementia have already been ongoing for many years. Biomarkers that can detect these processes before a clinical diagnosis can be made are strongly needed in order to identify persons who will develop dementia, to gain more insight in the pathogenesis of dementia and ultimately to help find new therapeutic agents that may alter or stop the disease. Currently, the most explored biomarkers are imaging markers and the proteins β -Amyloid (A β)-42 and tau that are altered in the cerebrospinal fluid of patients with Alzheimer disease early in the disease process.^{8,9} A lumbar puncture, required to obtain cerebrospinal fluid, is a relative invasive procedure that is not easily performed on large numbers of patients or in the general population, and imaging methods such as PET are not routinely available in all clinical settings. Less invasive biomarkers of dementia are therefore wanted.

The aim of this thesis is to search for non-invasive biomarkers and explore risk factors of dementia. All research is embedded in the Rotterdam Study, a large prospective population-based cohort study among people of 55 years and older in Ommoord, a district of Rotterdam, the Netherlands.¹⁰ The study started in 1990 and participants have been followed for occurrence of dementia and other diseases since. The cohort was extended in 2000 with persons who had become 55 years of age or moved into the study district since the start of the study, and again in 2006 with persons aged 45 years and older. Figure 1 shows the structure of the Rotterdam Study. The studies described in this thesis are based on participants that took part in the baseline examinations of the 1990 subcohort (chapters 2, 4, 6 and 7), the baseline examination

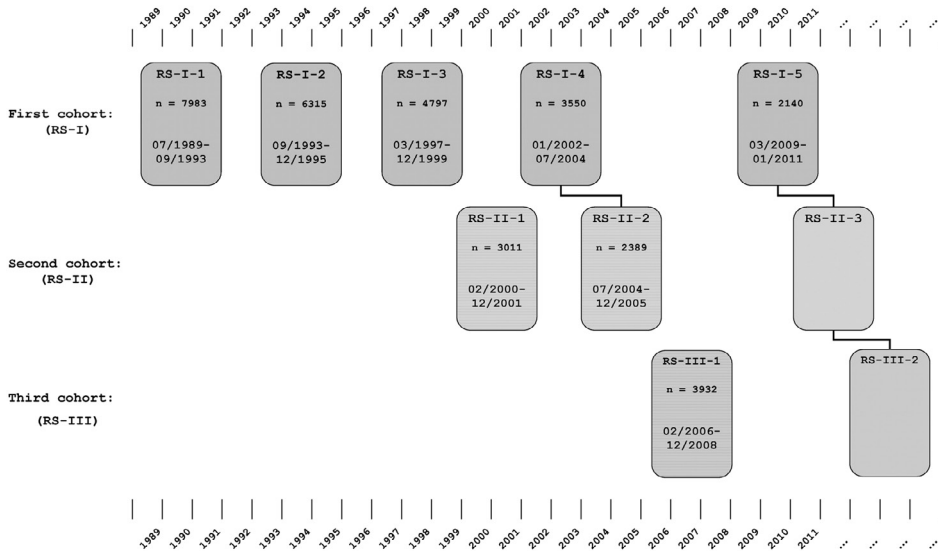


Figure 1. Diagram of the examination cycles of the Rotterdam Study (RS).

of the 2000 subcohort (chapter 2), or the third examination of the 1990 subcohort (chapters 3 and 5).

In projections of future dementia prevalence, typically stable incidence rates are presumed, and a possible effect of changes in prevention and treatment of vascular risk factors is not taken into account. **Chapter 2** describes the differences in dementia incidence rates, mortality rates and presence and treatment of vascular risk factors between the subcohort of the Rotterdam Study that started in 1990 and the subcohort that started in 2000.

Chapter 3 focuses on blood-based biomarkers of dementia. In **chapter 3.1**, I discuss a bias that is frequently introduced in studies and show that an incorrect selection of the study population can greatly influence the diagnostic performance of a biomarker. **Chapter 3.2** explores the use of plasma clusterin levels as a potential early biomarker of Alzheimer disease.

Chapter 4 focuses on retinal vascular abnormalities and their relation with dementia. In **chapter 4.1** the association between retinal vascular caliber and dementia is discussed and in **chapter 4.2**, I explore the relation of retinopathy and dementia.

Chapter 5 describes the associations of potential endocrine risk factors of dementia. In **chapter 5.1**, I show the relation of insulin metabolism with the risk of Alzheimer disease, and in **chapter 5.2**, the relation of serum cortisol with cognitive function, cognitive decline and the development of dementia is discussed.

Chapter 6 presents results of a genome-wide association study of vascular dementia.

In **chapter 7**, I explore which known risk factors contribute to the prediction of dementia, and what these factors add to age, the most important risk factor thus far. Finally, in **chapter 8**, I summarize our main findings and discuss implications and suggestions for future research.

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2

Incidence of dementia

Chapter 2

Is dementia incidence declining? Trends in dementia incidence since 1990 in the Rotterdam Study

Elisabeth M.C. Schrijvers, Benjamin F.J. Verhaaren, Peter J. Koudstaal,
Albert Hofman, M. Arfan Ikram, Monique M.B. Breteler

ABSTRACT

Background Vascular risk factors have been invoked in the etiology of late-life dementia. We hypothesized that changes in vascular risk factor profiles may have led to changes in age-specific incidence rates of dementia.

Methods We compared the incidence of dementia in two independent subcohorts of persons aged 60-90 years from the Rotterdam Study, a large population-based cohort study. The first subcohort started in 1990 (N=5727), the second in 2000 (N=1769). Participants were dementia-free at baseline and followed for at maximum five years. We calculated age-adjusted dementia incidence rates for the two subcohorts in total, in 10-years age strata, and for men and women separately. We also compared mortality rates, differences in prevalence of vascular risk factors and use of medication. Finally, we compared brain volumes and the extent of cerebral small vessel disease in participants who underwent brain imaging five years after the baseline examinations.

Results In the 1990 subcohort (25,696 person-years) 286 persons developed dementia, and in the 2000 subcohort (8,384 person-years) 49 persons. Age-adjusted incidence rates of dementia and mortality rates were lower in the 2000 subcohort compared to the 1990 subcohort in all strata. The prevalence of vascular risk factors increased between 1990 and 2000. This was paralleled by a strong increase in medication use for treatment of vascular risk factors. Participants in 2005-2006 had larger total brain volumes and less cerebral small vessel disease than participants in 1995-1996.

Conclusions Age-specific dementia incidence decreased between 1990 and 2005, possibly due to better treatment of vascular disease.

INTRODUCTION

The prevalence of dementia is increasing. Due to the aging of the population, the number of persons living with dementia worldwide is expected to double every 20 years with an expected number of 81 million people with dementia in 2040.¹ Future projections typically assume stable incidence rates of dementia, and do not take better prevention into account, which could lower the incidence and thereby cause a smaller rise in dementia prevalence.^{2,3} Studies investigating trends in dementia incidence have reported different results. Some have reported no change in incidence rates.⁴⁻⁷ Two studies that based the diagnosis of dementia solely on medical records reported increasing incidence rates, which will be at least in part a reflection of improved diagnostic procedures for dementia and an increase in case identification and diagnosis popularity.^{8,9} A recent paper reported decreasing incidence rates between 1985 and 1994.¹⁰ For stroke, incidence rates have declined over the past four decades in high income countries, presumably due to the implementation of preventive treatments and reduction in risk factors at the population level.^{11,12} Since vascular risk factors increase the risk for dementia,¹³ we hypothesized that incidence rates of dementia could have likewise declined.

To investigate whether dementia incidence changed over the last two decades, we compared the age-specific incidence rates of dementia and mortality in a cohort of elderly persons that started in 1990 with a cohort that started in 2000, both from the Rotterdam Study, a large population-based cohort study from the Netherlands.¹⁴ Furthermore, we compared brain volumes and the presence of cerebral small vessel disease between participants of both subcohorts who underwent a brain MRI five years after study entry.

METHODS

Study population

The Rotterdam Study started in 1990 and is conducted among all inhabitants aged 55 years and older of Ommoord, a district of Rotterdam, the Netherlands.¹⁴ The study was extended in 2000 with a new subcohort of persons who had become 55 years of age or moved into the study district since the start of the study. Follow-up examinations were repeated every three to four years. In addition, through linkage with records of general practitioners and the municipality, the total cohort was continuously monitored for morbidity and mortality. The medical ethics committee at Erasmus University of Rotterdam approved the study and written informed

consent was obtained from all participants. Details of the study have been described elsewhere.¹⁴

For the current study, we included all participants who were between 60 and 90 years, because of limited numbers of incident dementia under the age of 60, and limited numbers of participants older than 90.

Of the 6,485 participants between 60 and 90 in the 1990 subcohort, 6,100 participants were screened for dementia and prevalent dementia was diagnosed in 373 participants. In total, 5,727 participants were included in the analyses.

In the 2000 subcohort, of 1,992 participants between 60 and 90 years, 1,783 were screened for dementia and prevalent dementia was diagnosed in 14 participants. In total, 1,769 participants were included in the analyses.

Dementia case finding

Participants were screened for dementia at baseline and follow-up visits using a three-step protocol.¹⁵ Two brief tests of cognition (Mini-Mental State Examination (MMSE)¹⁶ and Geriatric Mental State schedule (GMS)¹⁷ organic level) were used to screen all participants. Screen-positives (MMSE score < 26 or GMS organic level > 0) underwent the Cambridge examination for mental disorders of the elderly.¹⁸ Participants 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 digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.¹⁵ The diagnosis of dementia was made in accordance with internationally accepted criteria (DSM-III-R)¹⁹ by a panel of a neurologist, neuropsychologist and research physician. The follow-up with regard to dementia diagnosis was virtually complete until January 1, 2007.

Baseline characteristics

Educational level was dichotomized into primary education (with or without an unfinished higher education) versus lower vocational to university education. Smoking habits were categorized as current, former and never cigarette smoking. History of stroke or myocardial infarction at baseline was verified by reviewing medical records.

Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in a sitting position. Hypertension was defined as a blood pressure $\geq 160/95$ or use of antihypertensive medication, prescribed for the indication of hypertension. The waist circumference was measured in centimeters.

Diabetes mellitus was defined as a self-reported history of diabetes, a random non-fasting or post-load serum glucose level ≥ 11.1 mmol/l (1990 subcohort), or a random fasting serum glucose level ≥ 7.0 mmol/l (2000 subcohort).

Brain imaging

Brain imaging was performed as part of the Rotterdam Scan Study.^{20,21} In 1995-1996, a random subset of the 1990 subcohort was invited to undergo brain imaging. From 2005 onwards, MRI imaging is routinely performed as part of the core examinations of the Rotterdam Study. Details of the study have been described elsewhere.^{20,21} In total, we had brain imaging data for 487 participants from the 1990 subcohort, and for 864 participants of the 2000 subcohort. All participants were between 60 and 90 years and free of dementia at the time of MRI-scanning which occurred five to six years after study entry.

In 1995-1996 brain MRI was performed on a 1.5-Tesla MRI System (VISION MR, Siemens AG) and included T1, proton-density and T2-scans.²⁰ In addition, a high-resolution T1, inversion-recovery, 3-D HASTE sequence was acquired. Slice thickness was 5 mm for T1, T2, and proton-density sequences, and 1.25 mm for the HASTE sequence. In 2005-2006 brain MRI was performed on a 1.5 Tesla scanner with an eight channel head coil (GE Healthcare) and included T1, proton-density, and fluid-attenuated inversion recovery (FLAIR) sequences.²¹ Slice thickness was 1.6 mm for the T1 and proton density sequences (zero-padded to 0.8mm for the T1 sequences), and 2.5 mm for the FLAIR sequence; all slices were contiguous.

Pre-processing steps, the segmentation algorithm, and validation results have been described previously.^{20,22} The brain tissue segmentation algorithm applied in 1995-1996 and 2005-2006 was the same, whereas the algorithm for the white matter lesions (WML) was slightly modified in 2005-2006 to incorporate the FLAIR images.²³

In 1995-1996 lacunar infarcts were rated visually as focal hyperintensities on T2-images, ≥ 3 mm in size. Hyperintensities in white matter also had to have corresponding prominent hypointensities on T1-images. Proton-density sequences were used to distinguish infarcts from dilated perivascular spaces.²⁰ In 2005-2006, lacunar infarcts were rated primarily on the FLAIR and proton-density sequences and defined as lesions ≥ 3 mm in size, exhibiting the same signal characteristics as cerebrospinal fluid on all sequences, and, if located supratentorially, with a hyperintense rim on the FLAIR sequence.²¹ In both subcohorts cortical infarcts were those infarcts showing involvement of gray matter, and persons with both lacunar and cortical infarcts were included in the group with cortical infarcts.²⁰

Statistical analyses

Baseline characteristics were compared between 1990 and 2000 per 10-years age strata, for men and women separately. Differences between the subcohorts were assessed using linear regression for continuous variables and logistic regression for dichotomous variables, adjusted for age. Fisher's exact test was used for dichotomous variables if a percentage was zero.

For the current study, participants contributed person-years for a maximum of five years after baseline. For the incidence of dementia, follow-up time was censored at date of dementia diagnosis, date of death, date of reaching the age of 90, or five years after baseline, whatever came first. Five-year follow-up was complete for more than 99% for both subcohorts.

Age-adjusted dementia incidence rates, mortality rates and incidence rate ratios (IRR) were calculated using Poisson regression models for the two subcohorts in total, in 10-years age-strata, and for men and women separately. An additional adjustment was made for age squared, to make sure the effect of age was adequately adjusted for.

Intracranial volume, total brain volume, and volume of WML on MRI were compared between the subcohorts in total, in 10-years age-strata, and for men and women separately, using ANOVA, adjusting for age. Total brain volume and WML were expressed as percentages of intracranial volume. Because of a non-normal distribution, WML were natural log transformed to compare the differences. Odds ratios for the presence of cortical and lacunar infarcts were calculated using logistic regression models, adjusted for age.

RESULTS

Baseline characteristics in strata of age and sex are presented in Table 1. Due to the design of the study, the age distribution was different in the 1990 subcohort compared to the 2000 subcohort. The distribution in the 2000 subcohort is more skewed towards younger participants who became eligible for participation only after the start of the first subcohort. In the 60-69 age stratum, both men and women were significantly younger in the 2000 subcohort. The 2000 subcohort was higher educated across all strata. Participants in the 2000 subcohort had higher blood pressure and more often hypertension, a higher body mass index and larger waist circumference, and had smoked more often before but were smoking less at present. More participants in the 2000 subcohort had diabetes and used antidiabetic medication, although the differences were mostly non-significant. The use of antithrombotics was three

Table 1. Baseline characteristics in strata of age and sex

	Subcohort	Age category					
		60-69		70-79		80-89	
		Men	Women	Men	Women	Men	Women
Number	1990	1241	1532	824	1259	248	623
	2000	553	636	194	255	50	101
Age (years)	1990	64.9 ± 2.8	64.9 ± 2.9	74.4 ± 2.9	74.6 ± 2.8	83.8 ± 2.7	84.1 ± 2.7
	2000	63.6 ± 2.8	63.1 ± 2.5	74.2 ± 2.8	74.7 ± 2.7	83.6 ± 2.4	83.6 ± 2.7
	P-value	<0.001	<0.001	0.45	0.46	0.57	0.06
Only primary education	1990	243 (20%)	576 (38%)	267 (33%)	623 (51%)	111 (48%)	395 (70%)
	2000	86 (16%)	164 (27%)	36 (19%)	99 (39%)	12 (24%)	40 (40%)
	P-value	0.23	<0.001	<0.001	<0.001	0.003	<0.001
Systolic blood pressure (mmHg)	1990	138 ± 21	136 ± 21	143 ± 23	145 ± 21	142 ± 23	151 ± 23
	2000	143 ± 21	141 ± 19	151 ± 23	155 ± 24	150 ± 24	163 ± 22
	P-value	<0.001	<0.001	<0.001	<0.001	0.03	<0.001
Diastolic blood pressure (mmHg)	1990	76 ± 11	74 ± 11	73 ± 12	73 ± 11	71 ± 12	73 ± 13
	2000	81 ± 10	78 ± 10	79 ± 11	78 ± 11	77 ± 13	77 ± 12
	P-value	<0.001	<0.001	<0.001	<0.001	0.002	0.01
Hypertension ≥160/95	1990	375 (31%)	504 (33%)	287 (37%)	564 (47%)	75 (35%)	293 (53%)
	2000	185 (36%)	194 (32%)	98 (51%)	139 (56%)	25 (51%)	70 (70%)
	P-value	0.02	0.46	<0.001	0.007	0.03	0.002
Body mass index (kg/m ²)	1990	25.8 ± 2.9	26.8 ± 4.0	25.7 ± 3.0	27.0 ± 4.1	24.7 ± 3.4	27.1 ± 4.2
	2000	27.0 ± 3.4	27.5 ± 4.5	26.5 ± 3.1	27.7 ± 4.4	25.5 ± 2.9	27.0 ± 3.9
	P-value	<0.001	<0.001	0.001	0.02	0.160	0.728
Waist circumference (cm)	1990	94.1 ± 9.1	87.1 ± 11.3	95.1 ± 9.7	88.8 ± 11.5	95.0 ± 10.7	92.1 ± 12.0
	2000	98.6 ± 9.8	89.5 ± 11.5	99.8 ± 9.3	91.7 ± 11.5	98.4 ± 8.4	91.8 ± 10.8
	P-value	<0.001	<0.001	<0.001	0.001	0.05	0.83
Smoking: Current	1990	322 (27%)	348 (23%)	167 (22%)	155 (13%)	54 (26%)	30 (5%)
	2000	119 (23%)	125 (20%)	24 (13%)	35 (14%)	1 (2%)	8 (8%)
	P-value	0.02	0.07	0.004	0.64	0.006	0.34
Smoking: Never	1990	100 (8%)	670 (45%)	103 (13%)	738 (61%)	40 (19%)	436 (75%)
	2000	94 (18%)	227 (37%)	22 (11%)	109 (43%)	7 (14%)	58 (57%)
	P-value	<0.001	0.01	0.49	<0.001	0.42	0.001
Diabetes	1990	121 (10%)	126 (8%)	105 (13%)	152 (12%)	38 (16%)	118 (20%)
	2000	55 (10%)	58 (9%)	35 (18%)	37 (15%)	9 (18%)	8 (8%)
	P-value	0.42	0.08	0.06	0.31	0.62	0.007
Myocardial infarction	1990	138 (11%)	34 (2%)	117 (15%)	56 (5%)	23 (10%)	39 (7%)
	2000	36 (7%)	13 (2%)	26 (13%)	6 (2%)	6 (12%)	7 (7%)
	P-value	0.01	0.52	0.69	0.11	0.63	0.88

Table 1. (continued)

	Subcohort	Age category					
		60-69		70-79		80-89	
		Men	Women	Men	Women	Men	Women
Stroke	1990	25 (2%)	18 (1%)	40 (5%)	26 (2%)	22 (9%)	37 (6%)
	2000	18 (3%)	10 (2%)	12 (6%)	6 (2%)	4 (8%)	7 (7%)
	P-value	0.10	0.20	0.44	0.79	0.84	0.66
Blood pressure lowering drugs	1990	348 (28%)	440 (29%)	279 (34%)	515 (41%)	92 (37%)	324 (52%)
	2000	138 (26%)	152 (24%)	76 (39%)	123 (48%)	22 (44%)	43 (43%)
	P-value	0.62	0.40	0.15	0.04	0.37	0.08
Antidiabetic therapy	1990	49 (4%)	47 (3%)	49 (6%)	82 (7%)	12 (5%)	49 (8%)
	2000	24 (5%)	22 (4%)	21 (11%)	20 (8%)	3 (6%)	3 (3%)
	P-value	0.30	0.13	0.02	0.46	0.75	0.08
Antithrombotics	1990	81 (7%)	38 (2%)	90 (11%)	45 (4%)	26 (11%)	39 (6%)
	2000	116 (22%)	39 (8%)	68 (35%)	63 (25%)	15 (30%)	31 (31%)
	P-value	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
Lipid lowering drugs	1990	45 (4%)	46 (3%)	13 (2%)	33 (3%)	0 (0%)	1 (0.2%)
	2000	76 (14%)	84 (14%)	35 (18%)	42 (16%)	1 (2%)	13 (13%)
	P-value	<0.001	<0.001	<0.001	<0.001	0.17*	<0.001

Data are presented as mean ± standard deviation or numbers (percentages).

Percentages are calculated without missing data. For all reported variables missings occurred in <5% of all participants.

Linear or logistic regression models, adjusted for age, were used to compare the differences

* Fisher's exact test, unadjusted

Table 2. Age-adjusted dementia incidence rates and incidence rate ratios of the 2000 versus the 1990 subcohort

Age stratum		Total	Men	Women
All	Incidence rate 1990	6.56	6.25	6.78
	Incidence rate 2000	4.92	4.48	5.20
	IRR (95% CI)	0.75 (0.56-1.02)	0.72 (0.44-1.16)	0.77 (0.52-1.14)
60-69 years	Incidence rate 1990	1.29	1.76	0.90
	Incidence rate 2000	1.08	1.39	0.82
	IRR (95% CI)	0.83 (0.29-2.41)	0.79 (0.20-3.08)	0.91 (0.17-4.94)
70-79 years	Incidence rate 1990	9.66	9.81	9.49
	Incidence rate 2000	6.36	4.69	7.82
	IRR (95% CI)	0.66 (0.40-1.10)	0.48 (0.21-1.11)	0.82 (0.43-1.56)
80-89 years	Incidence rate 1990	31.46	30.93	31.75
	Incidence rate 2000	26.42	30.41	24.22
	IRR (95% CI)	0.84 (0.56-1.26)	0.98 (0.51-1.90)	0.77 (0.45-1.29)

IRR= incidence rate ratio, CI= confidence interval

Incidence rates are per 1000 person-years

Incidence rates and IRR are adjusted for age

to six fold higher in the 2000 subcohort compared to the use in 1990 ($p < 0.001$ across all strata). Also, the use of lipid lowering drugs was much higher in the 2000 cohort ($p < 0.001$ across all strata, except men in the highest age stratum).

In the 1990 subcohort, after 25,696 persons-years, 286 incident dementia cases had occurred, and in the 2000 subcohort, 49 incident dementia cases had occurred after 8,384 person-years. Age-adjusted dementia incidence rates and IRR are shown in Table 2. The incidence of dementia was 25% lower in the 2000 subcohort compared to the 1990 subcohort, reaching borderline significance (IRR 0.75, 95% CI 0.56-1.02, $p = 0.06$). The dementia incidence was lower in the 2000 subcohort across all age strata. For men, the difference was largest in the 70-79 age stratum (IRR 0.48, 95% CI 0.21-1.11), while there was no difference in dementia incidence in the highest age stratum from 80-89 years. For women, the estimated reduction in dementia incidence increased from 9% in the lowest age stratum to 23% in the highest stratum. Further adjustment for age squared slightly changed the incidence rates, but not the IRR.

In the 1990 subcohort, 782 persons died of whom 709 without having a diagnosis of dementia. In the 2000 subcohort, 119 persons died, of whom 112 without dementia. Age-adjusted mortality rates and rate ratios are shown in Table 3. Overall, the age-adjusted mortality rate was 37% lower in the 2000 subcohort compared to the 1990 subcohort (RR 0.63, 95% CI 0.52-0.77, $p < 0.001$). For both men and women, the mortality rates were lower in the 2000 subcohort across all age strata. In men, the difference was most pronounced in the highest age stratum, where the mortality rate was 64% lower in the 2000 subcohort. In women, the difference was 68% in the

Table 3. Age-adjusted mortality rates and rate ratios of the 2000 versus the 1990 subcohort

Age stratum		Total	Men	Women
All	Mortality rate 1990	22.0	29.0	16.5
	Mortality rate 2000	14.0	18.5	9.7
	RR (95% CI)	0.63 (0.52-0.77)	0.64 (0.50-0.82)	0.59 (0.44-0.80)
60-69 years	Mortality rate 1990	11.1	16.4	6.7
	Mortality rate 2000	6.4	11.8	2.1
	RR (95% CI)	0.58 (0.38-0.88)	0.72 (0.45-1.17)	0.32 (0.13-0.77)
70-79 years	Mortality rate 1990	25.1	32.4	19.8
	Mortality rate 2000	21.7	28.4	15.7
	RR (95% CI)	0.87 (0.65-1.16)	0.88 (0.60-1.28)	0.79 (0.50-1.26)
80-89 years	Mortality rate 1990	69.3	109.1	50.8
	Mortality rate 2000	33.1	38.9	29.0
	RR (95% CI)	0.48 (0.34-0.67)	0.36 (0.21-0.60)	0.57 (0.36-0.89)

RR= rate ratio, CI= confidence interval
Mortality rates are per 1000 person-years
Mortality rates and RR are adjusted for age

Table 4. Age-adjusted mean brain volumes in 1995-1996 versus 2005-2006

		Age category							
		All		60-69		70-79		80-89	
	Year of MRI	Men	Women	Men	Women	Men	Women	Men	Women
Number	1995-1996	240	247	90	97	86	92	64	58
	2005-2006	425	439	320	354	87	67	18	18
		Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
Intracranial volume (ml)	1995-1996	1204 (6.7)	1064 (5.7)	1204 (10.3)	1065 (9.0)	1196 (10.5)	1060 (8.5)	1200 (12.4)	1045 (8.8)
	2005-2006	1189 (4.9)	1051 (4.2)	1197 (5.4)	1058 (4.7)	1176 (10.4)	1043 (10.0)	1169 (23.5)	1034 (15.9)
	P-value	0.10	0.09	0.52	0.49	0.20	0.21	0.25	0.53
Total brain volume (% of ICV)	1995-1996	78.2 (0.2)	79.0 (0.2)	79.5 (0.3)	80.4 (0.3)	76.9 (0.3)	77.5 (0.3)	73.9 (0.3)	74.1 (0.3)
	2005-2006	80.7 (0.1)	82.7 (0.1)	82.1 (0.2)	84.1 (0.2)	79.5 (0.3)	80.7 (0.3)	75.8 (0.6)	78.3 (0.6)
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.007	<0.001
WML volume (% of ICV)	1995-1996	0.83 (0.06)	1.34 (0.08)	0.57 (0.07)	1.05 (0.08)	1.04 (0.10)	1.45 (0.13)	1.60 (0.20)	2.95 (0.29)
	2005-2006	0.68 (0.05)	0.79 (0.06)	0.44 (0.04)	0.46 (0.04)	0.91 (0.10)	1.00 (0.15)	1.31 (0.38)	2.31 (0.52)
	P-value*	0.49	<0.001	0.62	<0.001	0.95	0.11	0.29	0.18

SE= standard error, WML= white matter lesions

* p-values based on analyses with WML natural log transformed

Means are adjusted for age, using ANOVA

youngest age stratum, 21% in the 70-79 stratum, and 43% in the highest age stratum. Further adjustment for age squared did not change the results.

Total brain volume was higher in 2005-2006 compared to 1995-1996 for both men and women in all age strata (p-values \leq 0.007) (Table 4). The WML volume was consistently lower in 2005-2006, though not always significantly so. In 1995-1996 cortical infarcts were present in 24 participants (5%) versus 19 participants (2%) in 2005-2006 (OR 2005-2006 versus 1995-1996: 0.75, 95% CI 0.38-1.49). We had insufficient power to test differences in strata. Lacunar infarcts were present in 112 participants (23%) in 1995-1996 and in 59 participants (7%) in 2005-2006 (OR 0.43, 95% CI 0.30-0.63). The difference was more pronounced in women (OR 0.19, 95% CI 0.10-0.34) than in men (OR 0.81, 95% CI 0.49-1.35). In women, the difference was significant and virtually the same across all age strata, whereas in men lacunar infarcts were less present in 2005-2006 compared to 1995-1996 in the age strata 60-69 and 70-79, but more present in 2005-2006 in the highest age stratum (data not shown). In men, these differences were all non-significant (p-values $>$ 0.40).

DISCUSSION

We found lower incidence rates of dementia in the 2000 subcohort than in the 1990 subcohort. Evidence for a declining trend in dementia incidence was further supported by the observation that participants from the 2000 subcohort had on average less brain atrophy and less cerebral small vessel disease on MRI. Our results are in line with a recent report on trends in the incidence and prevalence of dementia in the USA, where a decline in dementia incidence was observed in Rochester between 1985 and 1994.¹⁰ Earlier reports from Rochester reported no change in incidence between 1960 and 1974⁵ and a possible increase of dementia among persons of 85 years and older between 1965 and 1984.⁸ The reported increase, however, might very well be the result of an increasing awareness of dementia and an increased case identification over the years.⁸ The change in dementia incidence rates between 1985 and 1994 was a 3% decline per year, or a 30% decline in 10 years,¹⁰ which is in line with our overall reduction of 25% in 10 years. Interestingly, in the Chicago Health and Aging Project the effect estimate of annual change in dementia incidence between 1997 and 2008 was similar to the estimate found in Rochester, although not significant.^{4,10}

Although the reduction in dementia incidence rates in our study was also not significant at the conventional $\alpha=0.05$ level, we think our estimates reflect a true decline in incidence rates. First, the risk reduction was consistent across all strata of gender and age, but for men above 80 years. Second, we also found a clear reduction in mortality rates. A person who dies, is no longer at risk of developing dementia, while underlying risk factors, especially vascular factors, are associated with both dementia and mortality risk. The mortality rate, and thereby the competing risk effect,²⁴ was higher in the 1990 subcohort. Should mortality rates have been the same in both subcohorts, the difference in incidence rates of dementia would probably have been larger. Third, we used the same methods of diagnosing dementia for both subcohorts, and do not think that we underestimated the incidence in the 2000 subcohort. However, awareness and reporting of dementia by physicians have increased over the last decades. To the extent that this has influenced our study, it will have led to an increased case identification in the 2000 subcohort, and consequently an underestimating of the difference in incidence rates between both subcohorts. Finally, the larger brain volumes and lower presence of cerebral small vessel disease we observed comparing scans of non-demented participants made in 1995-1996 with scans made in 2005-2006, support our finding of declining dementia incidence. Larger brain volumes suggest less brain atrophy, and brain atrophy and cerebral small vessel disease are associated with a higher risk of dementia.²⁵

There are several possible explanations for our observation of a decreasing incidence of dementia. First, the 2000 subcohort was higher educated and higher education has

been associated with a later onset of dementia.^{26,27} However, the level of education may not adequately reflect the cognitive abilities of a person, when comparing two different birth cohorts. The older cohort probably had fewer possibilities to continue education after primary school, regardless of their intellectual abilities.

A second explanation, as proposed for the decreasing incidence of stroke, is the implementation of preventive treatments and reduction in vascular risk factors at the population level.^{11,12} In our study, however, apart from current smoking, vascular risk factors were more prevalent in the 2000 subcohort compared to the 1990 subcohort. This was, however, paralleled by a strong increase in use of antithrombotic and lipid-lowering drugs. Use of statins has been associated with a lower risk of dementia in our cohort²⁸ and others,^{29,30} and both antithrombotics and lipid-lowering drugs are preventive treatments for cerebrovascular disease.³¹ Our observation of less brain atrophy and small vessel disease on the MRI scans in the 2000 subcohort supports the notion that a better treatment of vascular risk factors could explain the decrease in dementia incidence.

The hypothesis that treatment of vascular factors might lower dementia risk has become more popular in recent years,³² but thus far, dementia has mostly been investigated as a secondary endpoint in clinical trials that were underpowered to assess a moderate reduction in dementia risk. However, a meta-analysis suggested a 13% risk reduction by antihypertensive treatment,³³ which is an effect size not unlike many other effects seen in the cardiovascular field.³⁴

Finally, a decline in stroke incidence itself could also attribute to a decreasing incidence of dementia, because stroke is associated with a higher risk of dementia independent of other vascular risk factors.³⁵

Important strengths of our study are the comparison of two large independent subcohorts from one population-based study, the equal assessment of dementia and vascular risk factors in both subcohorts and the virtually complete follow-up for both subcohorts. The Dutch health care system and a close collaboration with the general practitioners allowed for a continuous monitoring for incident dementia through medical records, even when participants did not participate in the follow-up visits. We had a limited number of dementia cases in the 2000 subcohort, where the age distribution was more skewed towards younger people. We accounted for this difference in age distribution by adjusting for age, additional adjustments for age squared and by analyzing in different age strata. The fact that we had only 49 cases in the 2000 subcohort not only limited our statistical power, but also precluded us looking at different subtypes of dementia. Finally, despite similar post-processing, we used different MRI-scanners in 1995-1996 and 2005-2006, which could have affected the comparison. We consider it unlikely, however, that this would have led to a consistent overestimation of total brain volume and at the same time a

consistent underestimation of WML and lacunar infarcts in 2005-2006 compared to 1995-1996.

Our study suggests that preventive interventions may be effective in at least moderately reducing the dramatic rise in absolute numbers of people living with dementia that have been projected for the coming decades.

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3

Blood-based biomarkers of dementia

Chapter 3.1

Biomarker profiles for Alzheimer disease:
diagnosing disease or establishing health?

Elisabeth M.C. Schrijvers, Albert Hofman, Peter J. Koudstaal, Monique M.B. Breteler

ABSTRACT

Background: The approach to biomarker discovery and evaluation lacks standard guidelines. An inappropriate selection of study cases and controls can bias the performance of a diagnostic test. We illustrate the impact of different control selections in the evaluation of a subset of a proposed biomarker panel for Alzheimer disease.

Methods: The study was based on 61 persons with Alzheimer disease and 952 participants without dementia who were all selected from the Rotterdam Study, a population-based cohort study. We used a subset of 8 of the 18 proteins from a proposed biomarker panel to predict disease status in the original online data in which the panel was discovered, in our total study population and with two differently selected control groups. For the first control group we selected 61 controls frequency matched on age and sex, and for the second control group we selected 61 younger and very healthy controls to inflate the differences between the patients and the controls.

Results: The panel identified Alzheimer patients with 83% accuracy in the original discovery dataset. In our total population the panel could not distinguish Alzheimer patients from the non-demented controls. With the selected matched control group, the panel correctly classified half of the Alzheimer patients, which was no better than by chance. When we included only very healthy controls, the panel did better with an overall accuracy of 70%.

Conclusion: We demonstrated that different control selections can highly influence the accuracy of a diagnostic panel. An inflated contrast between patients and controls due to the selection of healthy controls can lead to a high accuracy, which does not reflect the real performance in a clinical setting.

INTRODUCTION

Extensive research efforts are directed on finding tools that can help in the early diagnosis of Alzheimer disease.¹ Early diagnosis of Alzheimer disease is difficult and only when the cognitive deficits become severe enough to interfere with normal daily functioning, the clinical diagnosis of Alzheimer disease will be made.² The differential diagnosis between Alzheimer disease and other types of dementias can also be difficult and a diagnostic tool that can detect Alzheimer disease in an early stage and distinguish it from other dementias is wanted.³ Biomarkers can help the detection and monitoring of disease⁴, and a lot of research is focused on finding plasma or CSF biomarkers for Alzheimer disease.¹

While the development of new clinical treatments adheres to well established standards for a phased approach for the discovery and evaluation of such treatments, the approach to biomarker discovery and evaluation thus far lacks similar standard guidelines. To avoid bias it has been proposed that discovery studies should use randomized selection of case patients and control subjects from a well-defined prospective cohort, that is relevant to the clinical application.^{5,6}

To emphasize the importance of the selection of an appropriate study population we assessed the impact of different control selections from the general population, using a subset of a blood-based biomarker panel that was developed in a clinical setting. In that setting, the panel could distinguish Alzheimer patients from healthy controls and other dementias with 89% accuracy.⁷ The panel was also able to predict progression from mild cognitive impairment (MCI) to Alzheimer disease several years before the clinical diagnosis was made with 81% accuracy.⁷ Subsequent research with the original data showed that a smaller subset of 5 of the 18 proteins had a similar performance as the total panel.⁸ However, the panel was developed in a setting with moderate Alzheimer patients and healthy, younger controls with a high average score on the Mini Mental State Examination (MMSE).⁷ These differences, and the high prior probability in a setting with equal numbers of cases and controls are likely to influence the accuracy of the panel.⁴

We assessed the impact of different control selections on the performance of the biomarker panel in participants from the Rotterdam Study, a large population-based cohort study.

METHODS

Study population

We first assessed the performance of our subset of 8 of the 18 proteins from the biomarker panel in the original dataset in which the panel was developed, which is available online.⁷ For evaluation of the panel we used participants of the Rotterdam Study, a large prospective population-based cohort study that is conducted among all inhabitants aged 55 years and over of Ommoord, a district of Rotterdam, the Netherlands.⁹ We selected a random subset of 952 participants that were free of dementia and 61 participants with prevalent Alzheimer disease, who all participated in the 1997-1999 examination.

To increase the prior probability of having Alzheimer disease to 50%, the same as in the discovery setting, we selected two different control groups from the non-demented controls with an equal number as the cases. First, we selected a group with 61 age and sex matched controls, and second, a group with 61 very healthy controls to increase the differences between cases and controls. For the latter we selected controls who did not use any medication, and had a MMSE-score of ≥ 29 .

Plasma measurements

Fasting blood samples were obtained at the research center and 5 ml of citrate plasma was collected and stored at -80°C . In July 2008, 200 μl of citrate plasma from each participant was sent to Rules Based Medicine, Austin, TX, USA, (www.rulesbased-medicine.com), where multianalyte profiling assays were performed using an xMAP technology. As part of a larger study, where we measured 147 different analytes, we measured 13 of the 18 proteins that were part of the original reported panel.⁷ In 5 of these proteins a large number of data was missing due to a low signal. These 5 were left out of the analyses. This resulted in a protein panel consisting of 8 proteins from the original reported panel: ANG-2, ICAM-1, IL-8, M-CSF, PDGF, PARC, RANTES, and TNF- α .

Dementia case finding

The diagnosis of dementia was made following a three-step protocol.¹⁰ Two brief tests of cognition (MMSE¹¹ and Geriatric Mental State schedule (GMS)¹² organic level) were used to screen all participants. Screen-positives (MMSE score < 26 or GMS organic level > 0) underwent the Cambridge examination for mental disorders of the elderly (Camdex).¹³ Participants 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 digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.¹⁰ The diagnoses of dementia and Alzheimer disease were made in accordance with internationally accepted criteria for dementia (DSM-III-R),¹⁴ and Alzheimer disease (NINCDS-ADRDA)¹⁵ by a panel of a neurologist, neuropsychologist and research physician.

Other covariates

Educational level was assessed during the baseline interview that took place between 1990 and 1993 and dichotomized into primary education (with or without a higher not completed education) versus lower vocational to university education. *APOE* genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis. *APOE* $\epsilon 4$ -status was defined as carriage of one or two $\epsilon 4$ -alleles.

Statistical Analysis

We used linear regression analyses to assess whether age and sex were significantly associated with the levels of the 8 different proteins and thus might act as confounders.

To predict disease status we performed logistic regression analysis with the panel of the 8 proteins combined. For internal validation we performed leave-one-out cross validation. First, we used the combination of the 8 proteins to predict Alzheimer disease in the original online data to compare the performance of the panel to the original panel of 18 proteins. Next, we performed Z score transformation on our data and used the estimates derived from the original online data to predict Alzheimer disease in our sample from the Rotterdam Study.

We then used estimates derived from our own data to predict disease status in our data; first with the total study population, then with the matched control group and finally with the healthy control group. We compared the last prediction with the prediction based on four known risk factors; age, sex, *APOE* $\epsilon 4$ -status and educational level.

All predictions, except for the prediction based on the estimates derived from the original online data, were done using the Weka software package version 3.6.0, which is available online (<http://www.cs.waikato.ac.nz/~ml/weka>). The linear regression analyses and the prediction in our data based on the estimates derived from the original online data were done using SPSS 15.0 (SPSS Inc., Chicago, Ill).

RESULTS

Baseline characteristics of the Alzheimer patients and different control groups from the Rotterdam Study are shown in table 1. All protein levels except for ICAM-1 varied significantly with age and all, except for RANTES and TNF- α , were significantly associated with sex (adjusted for age). Based on the online original data, the combination of the 8 proteins could predict Alzheimer disease with 83% accuracy (Figure 1). First, we used the estimates that we obtained from the original data to classify our participants from the Rotterdam Study. These estimates are derived from a setting in which the prior probability of having Alzheimer disease was around 50%. With these estimates, regardless of their actual case status, one third of our participants were classified as case (30% of the Alzheimer patients and 34% of the controls). Second,

Table 1. Baseline characteristics of the study population

	Alzheimer patients N=61	All controls N=952	Matched controls N=61	Healthy controls N=61
Age (yrs)	83.3 \pm 7.3	72.8 \pm 7.3	82.5 \pm 6.6	69.4 \pm 5.7
Female %	67	55	67	49
MMSE	18.8 \pm 5.2	27.7 \pm 1.9	26.8 \pm 2.2	29.4 \pm 0.5
APOE ϵ 4-allele present %	59	29	28	30
Low education %	64	28	48	18
History of hypertension %	57	52	62	15
Diabetes %	25	13	15	7
No. of medications	3.3 \pm 2.3	2.7 \pm 2.3	3.2 \pm 2.5	0

Data are presented as mean \pm standard deviation or percentages

	Trainingset n=83		Testset 'AD' n=92		
	AD	NDC	AD	NDC	OD
Clinical diagnosis	43	40	42	39	11
Classified as AD	36	10	37	6	2
Classified as non-AD	7	30	5	33	9
Percent agreement with clinical diagnosis	84% positive	75% negative	88% positive	84% negative	
83% overall agreement					

Figure 1. Classification of Alzheimer patients and controls in the original dataset from Ray et al.⁷ based on a subset of 8 plasma proteins

Classifications are based on a logistic model with 8 plasma proteins: ANG-2, ICAM-1, IL-8, M-CSF, PDGF, PARC, RANTES and TNF- α
AD = Alzheimer disease, NDC = non-demented control, OD = other dementia

we derived estimates from our own data. In this dataset the prevalence of Alzheimer disease and thus the prior probability was 6%. With these estimates, only one case and two controls were classified as Alzheimer patient (Figure 2a).

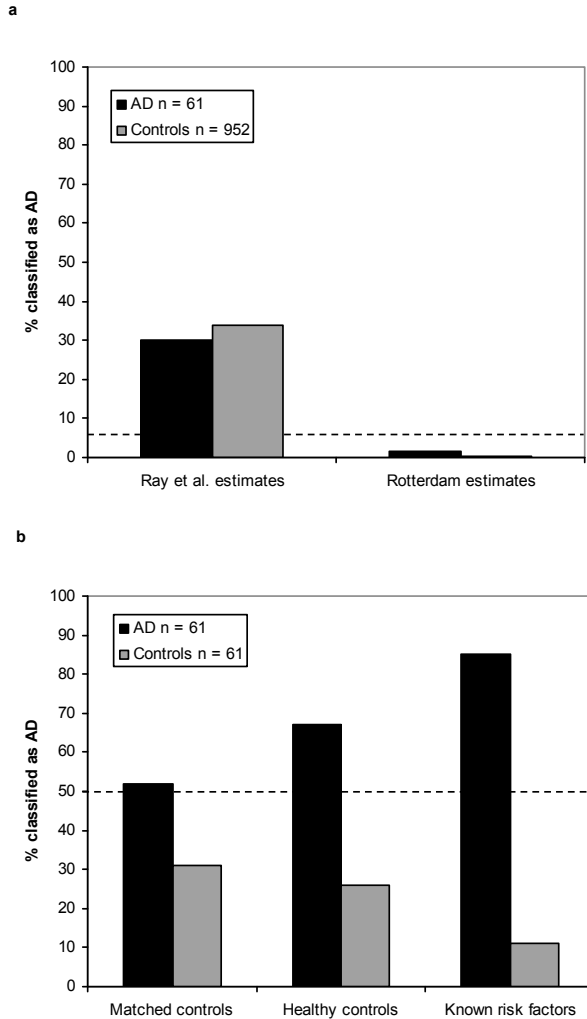


Figure 2. Percentage of people classified as having Alzheimer disease using different estimates and control groups

a. Population setting with a prior probability of Alzheimer disease of 6%. All classifications are based on a logistic model with 8 plasma proteins: ANG-2, ICAM-1, IL-8, M-CSF, PDGF, PARC, RANTES and TNF- α . The first classification is based on the estimates derived from a logistic regression analysis of the original data from Ray et al. The second classification is based on a logistic regression analysis of our own data.

b. Setting with a prior probability of Alzheimer disease of 50%: 1. with 61 age and sex frequency matched controls 2. with 61 healthy controls, all selected from the population cohort. The first two classifications are based on a logistic model with the 8 plasma proteins with the two different control groups. The third classification is based on a model with the known risk factors age, sex, *APOE* ϵ 4-status and educational level to classify between the Alzheimer patients and the healthy controls.

---The dotted line indicates the prior probability of Alzheimer disease

AD = Alzheimer disease

When we mimicked a case-control setting using the same cases and randomly selected 61 controls, frequency matched on age and sex, 42 controls (69%) were correctly classified, but only 32 cases (52%) were accurately classified as Alzheimer patients, which is nearly the prior probability.

After inflating the differences between cases and controls by selecting 61 healthy, younger controls with a high score on the MMSE, the classification improved to 67% of Alzheimer cases being correctly classified (true positives) and 74% of controls (true negatives). However, a model with the four known risk factors age, sex, *APOE* ϵ 4-status and educational level performed better and was able to correctly classify 52 cases (85%) and 54 healthy controls (89%) (Figure 2b).

DISCUSSION

In this study we showed that the selection of different controls can greatly influence the accuracy of a diagnostic test. After inflating the differences between patients and controls, a panel that has no real discriminative abilities in a population setting, seemed to perform quite well with a diagnostic accuracy of 70%.

In the search for a diagnostic test for Alzheimer disease we should keep in mind what such a test should be able to do. We do not need a tool that can distinguish Alzheimer patients from healthy, younger controls. A real reliable diagnostic test should be able to identify Alzheimer patients in an elderly population.

Ideally, biomarkers should be discovered and tested in a context that is representative for the envisioned situation in which they will be used. However, quite often diagnostic test are developed in a setting with large differences between cases and controls. In case of the biomarker panel we used to illustrate our message, the panel was developed in a setting where both the non-demented controls and the controls with dementias other than Alzheimer disease, were years younger than the Alzheimer patients.⁷ We showed that age significantly influenced the levels of 7 of the 8 proteins. Given that the incidence of Alzheimer disease exponentially increases with age, it seems likely that this age difference has influenced the reported high accuracy of the panel.

Because we had a subset of only 8 of the 18 proteins from the proposed biomarker panel and we used a different technology to measure the biomarkers, we can not use our data as conclusive evidence that the proposed panel is not a valid diagnostic tool for Alzheimer disease. However, since we clearly show the effect of inflating the differences between patients and controls on the diagnostic accuracy of our panel and the fact that the combination of the 8 proteins has an accuracy of 83% in the original data that are published online, it seems likely that the reported high accuracy reflects

an inflated contrast between patients and controls, rather than an Alzheimer specific protein profile. This is supported by another study that tried to replicate the protein panel and found that the panel could not accurately differentiate Alzheimer patients from MCI patients or healthy controls.¹⁶

In conclusion, we showed that an inappropriate selection of a control group can greatly bias the accuracy of a diagnostic tool and differences between patients and controls other than the specific disease in question should be taken into account in the discovery and evaluation of such a tool.

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Chapter 3.2

Plasma clusterin and the risk of Alzheimer disease

Elisabeth M.C. Schrijvers, Peter J. Koudstaal, Albert Hofman, Monique M.B. Breteler

ABSTRACT

Context Variants in the clusterin gene are associated with the risk of Alzheimer disease and clusterin levels have been found to be increased in brain and cerebrospinal fluid of patients with Alzheimer disease. Plasma clusterin was reported to be associated with brain atrophy, baseline disease severity, and rapid clinical progression in patients with Alzheimer disease.

Objective To evaluate the potential of plasma clusterin as a biomarker of the presence, severity, and risk of Alzheimer disease.

Design, Setting, and Participants A case-cohort study nested within the Rotterdam Study, a prospective population-based cohort study conducted in Rotterdam, the Netherlands. Plasma levels of clusterin were measured at baseline (1997-1999) in 60 individuals with prevalent Alzheimer disease, a random subcohort of 926 participants, and an additional 156 participants diagnosed with Alzheimer disease during follow-up until January 1, 2007 (mean [SD], 7.2 [2.3] years).

Main Outcome Measures Prevalent Alzheimer disease, severity of Alzheimer disease measured by the Mini-Mental State Examination (MMSE) score, and the risk of developing Alzheimer disease during follow-up.

Results The likelihood of prevalent Alzheimer disease increased with increasing plasma levels of clusterin (odds ratio [OR] per SD increase of plasma clusterin level, 1.63; 95% confidence interval [CI], 1.21-2.20; adjusted for age, sex, education level, apolipoprotein E status, diabetes, smoking, coronary heart disease, and hypertension). Among patients with Alzheimer disease, higher clusterin levels were associated with more severe disease (adjusted difference in MMSE score per SD increase in clusterin levels, -1.36; 95% CI, -2.70 to -0.02; $P=.047$). Plasma clusterin levels were not related to the risk of incident Alzheimer disease during total follow-up (adjusted HR, 1.00; 95% CI, 0.85-1.17; P for trend=.77) or within 3 years of baseline (adjusted HR, 1.09; 95% CI, 0.84-1.42; P for trend=.65).

Conclusion Plasma clusterin levels were significantly associated with baseline prevalence and severity of Alzheimer disease, but not with incidence of Alzheimer disease.

INTRODUCTION

Several genome-wide association studies have identified the *CLU* gene, which encodes for clusterin, as a genetic locus involved in Alzheimer disease.¹⁻³ The protein clusterin, also known as apolipoprotein J, has been suggested to be involved in the pathogenesis of Alzheimer disease.^{4,5} Clusterin has been found in the frontal cortex and hippocampus of postmortem Alzheimer disease brains⁶ and is increased in the cerebrospinal fluid of patients with Alzheimer disease.⁷ Plasma clusterin was reported to be associated with brain atrophy, baseline disease severity, and rapid clinical progression in Alzheimer disease, suggesting its possible use as a biomarker of Alzheimer disease.⁸ We used data from a large population-based cohort study to examine the associations between plasma levels of clusterin and the prevalence, severity, and risk of Alzheimer disease.

METHODS

Study Population

This study was based on participants of the Rotterdam Study, a large prospective population-based cohort study that is conducted among all inhabitants aged 55 years or older of Ommoord, a district of Rotterdam, the Netherlands.⁹ Baseline examinations were conducted between 1990 and 1993, with follow-up examinations conducted in 1993-1994, 1997-1999, and 2002-2004. The medical ethics committee at Erasmus University of Rotterdam, Rotterdam, the Netherlands, approved the study, and written informed consent was obtained from all participants.

This study was based on participants that took part in the third survey (1997-1999). Of the 5990 individuals alive at the time of the third survey, 4797 participated in the survey. A total of 3795 participants had fasting blood samples drawn that could be used for clusterin assessment. Among these participants, 79 were diagnosed with prevalent dementia and 7 did not undergo dementia screening, resulting in a cohort of 3709 participants at risk for incident dementia.

Diagnosis of dementia

At each survey, the diagnosis of dementia was made following a 3-step protocol.¹⁰ Two brief tests of cognition (Mini-Mental State Examination [MMSE]¹¹ and Geriatric Mental State schedule (GMS)¹² organic level) were used to screen all participants. Participants who screened positive (an MMSE score of <26 or GMS organic level of

>0) underwent the Cambridge Examination for Mental Disorders of the Elderly.¹³ Participants who were suspected of having dementia were examined by a neuropsychologist, if necessary. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.¹⁰ The diagnoses of dementia and its subtypes were made in accordance with internationally accepted criteria for dementia (DSM-III-R),¹⁴ Alzheimer disease (NINCDS-ADRDA),¹⁵ and vascular dementia (NINDS-AIREN)¹⁶ by a panel of a neurologist, neuropsychologist, and research physician. Follow-up for incident dementia was virtually complete (>98%) through January 1, 2007.

Study Design

We used a case-cohort study design, which is an established method that increases efficiency, especially when costly measurements are required.¹⁷ In this study design, a random subcohort is drawn from the total cohort at risk. Participants from the total cohort who develop the disease outside the subcohort are added to the analyses; however, only persons from the subcohort contribute follow-up time. In our study, the total cohort at risk for dementia consisted of 3709 persons. From this cohort, we drew a random subcohort of 952 participants in 2008, of whom 926 had sufficient plasma remaining for clusterin measurement. As of follow-up through January 1, 2007, we identified 61 participants who developed dementia in this subcohort (of whom 52 were diagnosed with Alzheimer disease) with clusterin measurement and 178 participants who developed dementia in the rest of the cohort (of whom 156 were diagnosed with Alzheimer disease), resulting in 237 incident dementia cases in the analysis (2 incident dementia cases did not have enough plasma available for measurement of clusterin). Because we wanted to investigate the associations of plasma clusterin levels with both prevalent and incident dementia and Alzheimer disease, we also measured clusterin levels in the 77 patients with prevalent dementia with sufficient plasma for measurement.

Assessment of Clusterin

At the third survey, fasting blood samples were obtained at the research center. Citrate plasma (5 mL) was collected and stored at -80°C . In July 2008, 200 μL of citrate plasma from each participant was sent to Rules-Based Medicine, Austin, Texas (www.rulesbasedmedicine.com), where clusterin levels were analyzed via multiplex immunoassay on a human multianalyte profile. The least detectable dose was 1.3

µg/mL. The intra-assay variability was less than 4% and the interassay variability was less than 13%.

Covariates

Educational level was assessed during the first interview, which took place between 1990 and 1993, and was dichotomized into primary education (with or without an unfinished higher education) versus lower vocational to university education. *APOE* (apolipoprotein E) genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis. *APOE* $\epsilon 4$ status was defined as carriership of 1 or 2 $\epsilon 4$ alleles. If *APOE* genotype was missing (n=42, 4.3%), *APOE* $\epsilon 4$ status was imputed as 0.28 (the proportion with an *APOE* $\epsilon 4$ allele in the total population with *APOE* genotyping). The MMSE score¹¹ was assessed at the research center during the third survey. In addition, a dedicated neuropsychological test battery was used to assess executive function, attention, and information processing speed. The test battery included the Letter-Digit Substitution Task,¹⁸ the Word Fluency Test,¹⁹ and the abbreviated Stroop test.²⁰

Hypertension was defined as a blood pressure of at least 140/90 mm Hg or use of antihypertensive medication, prescribed for the indication of hypertension. Coronary heart disease was defined as a previous myocardial infarction, percutaneous transluminal coronary angiography, or coronary artery bypass graft surgery. Smoking habits were assessed at the home interview. Diabetes was defined as a self-reported history of diabetes, registration by a general practitioner as having diabetes, or a fasting glucose level of at least 7.0 mmol/L. Missing values in covariates (<5%) were imputed as the mean.

Statistical Analyses

We used linear regression analyses to investigate the associations between the baseline characteristics and plasma clusterin levels. Analyses were adjusted for age and sex when applicable. All analyses were performed using SPSS statistical package 15.0 (SPSS Inc, Chicago, Illinois) or SAS version 9.2 (SAS Institute Inc, Cary, North Carolina). A priori level of significance was set at a p-value \leq 0.05 for all analyses.

First, we investigated the cross-sectional association between plasma levels of clusterin and prevalent Alzheimer disease and dementia using logistic regression models. After establishing that clusterin followed a normal distribution, clusterin was entered continuously per SD increase into the models and per quartile of its distribution. All analyses were adjusted for age and sex, and additional adjustments were made for educational level, *APOE* $\epsilon 4$ status, and vascular risk factors.

Second, to test whether plasma clusterin levels are associated with severity of Alzheimer disease within individuals with prevalent Alzheimer disease, we performed linear regression analyses of clusterin levels with the MMSE score and other cognitive test scores as the dependent variable.

Third, we investigated the association between plasma clusterin and the risk of developing incident Alzheimer disease during follow-up using Cox proportional hazards regression models with modification of the standard errors based on robust variance estimates. We used the method according to Barlow in which the random subcohort is weighted by the inverse of the sampling fraction from the total cohort at risk.¹⁷ All analyses were adjusted for age (used as the time scale) and sex, and additional adjustments were made for the abovementioned covariates.

In addition, to see whether plasma clusterin levels might have changed due to sub-clinical Alzheimer disease, subsequent analyses were performed on incident cases identified within and after 3 years of follow-up.

RESULTS

Baseline characteristics of the source population, the subcohort, and the prevalent Alzheimer cases are shown in Table 1. The random subcohort with plasma clusterin measurements did not differ from the total cohort at risk. Mean follow-up time was 7.2 years (SD, 2.3 years; range, 0.1-9.7 years). Of the 77 prevalent dementia cases, 60

Table 1. Baseline characteristics of the study population

	Total cohort at risk N=3709	Subcohort at risk N=926	Prevalent AD N=60
Age (years)	72.1 ± 6.8	72.8 ± 7.3	83.4 ± 7.3
Women, No. (%)	2133 (58)	508 (55)	41 (68)
<i>APOE</i> ε4-allele present, No. (%)	980 (28)	253 (28)	32 (58)
Education, only primary, No. (%)	1097 (30)	259 (28)	39 (65)
Current smoking, No. (%)	589 (16)	130 (14)	4 (8)
Coronary heart disease, No. (%)	386 (10)	96 (10)	9 (15)
Diabetes, No. (%)	514 (14)	118 (13)	15 (25)
Hypertension, No. (%)	2772 (76)	705 (78)	48 (86)
MMSE (points)	27.7 ± 1.9	27.7 ± 1.9	18.7 ± 5.2
Clusterin (µg/ml)	-	115 ± 25	129 ± 29

AD= Alzheimer disease

Data are presented as mean ± standard deviation or numbers (percentages).

Percentages are calculated without missing data. For all reported variables missings occurred in ≤5% of all participants.

Hypertension was defined as a blood pressure ≥ 140/90 or use of anti-hypertensive medication, prescribed for the indication of hypertension.

Table 2. Associations of baseline characteristics with plasma clusterin levels ($\mu\text{g/ml}$)

	Difference in clusterin levels (95% CI)	P-value
Age per year	-0.15 (-0.37; 0.07)	0.18
Female gender, yes/no	11.1 (8.00; 14.2)	<0.001
<i>APOE</i> ϵ 4-allele present, yes/no	0.57 (-2.94; 4.09)	0.75
Education, only primary, yes/no	-0.72 (-4.28; 2.83)	0.69
Current smoking, yes/no	-0.70 (-5.21; 3.82)	0.76
Coronary heart disease, yes/no	-1.94 (-7.11; 3.24)	0.46
Diabetes, yes/no	3.77 (-0.89; 8.43)	0.11
Hypertension, yes/no	5.29 (1.43; 9.15)	0.01

Linear regression analyses were performed in the random subcohort, $N=926$, and are adjusted for age and sex, when applicable

were diagnosed with Alzheimer disease, 9 with vascular dementia, and 8 with other types of dementia. Of the 237 incident dementia cases, 208 were diagnosed with Alzheimer disease (of whom 76 were diagnosed within 3 years of baseline), 20 with vascular dementia, and 9 with other types of dementia. Associations of the baseline characteristics with plasma clusterin levels are shown in Table 2.

Tables 3 and 4 show the associations of plasma clusterin levels with prevalent Alzheimer disease and with the risk of incident Alzheimer disease during follow-up. The odds that a participant had prevalent Alzheimer disease significantly increased by 49% for every SD increase in clusterin levels. This association became even stronger after further adjustments for educational level, *APOE* ϵ 4 status, and vascular factors. There was no statistically significant association of plasma clusterin levels with incident Alzheimer disease during total follow-up or with incident Alzheimer disease within or after 3 years of baseline. Results for all-cause dementia and vascular dementia were similar and are shown in Table 5.

After adjusting for age and sex, clusterin levels were associated with the MMSE score in patients with prevalent Alzheimer disease (difference in MMSE score per SD increase in clusterin levels, -1.34; 95% confidence interval [CI], -2.54 to -0.13; $P=.03$), but not in controls without dementia (difference in MMSE score per SD increase in clusterin levels, -0.004; 95% CI, -0.128 to 0.120; $P=.95$). Adjusting for education level, *APOE* ϵ 4 status, smoking, diabetes, coronary heart disease, and hypertension did not change the results (difference in MMSE score per SD increase in clusterin levels, -1.36; 95% CI, -2.70 to -0.02; $P = .047$ for patients with prevalent Alzheimer disease and -0.005; 95% CI, -0.126 to 0.116; $P =.93$ for controls without dementia). A smaller subset underwent additional cognitive tests (Letter-Digit Substitution Task, Word Fluency Test, and Stroop test), which largely showed the same pattern but did not reach statistical significance (Table 6).

Table 3. Odds ratios of prevalent Alzheimer disease per standard deviation increase and per quartile in plasma clusterin levels

Clusterin ($\mu\text{g/ml}$)	Prevalent Alzheimer disease at baseline n=60	
	OR (95% CI) ¹	OR (95% CI) ²
Per SD	1.49 (1.12-1.98)	1.63 (1.21-2.20)
Per Quartile		
47.2 -99.5	1.00 (ref)	1.00 (ref)
99.6-115	0.98 (0.38-2.45)	0.99 (0.36-2.78)
116-132	1.73 (0.70-4.25)	2.17 (0.83-5.68)
133-198	2.32 (1.03-5.26)	2.99 (1.25-7.16)
P-trend	0.02	0.004

OR=odds ratio, n=number of Alzheimer cases

¹ adjusted for age and sex² adjusted for age, sex, education, *APOE* ϵ 4-carriership, diabetes, smoking, coronary heart disease and hypertension**Table 4.** Risk of incident Alzheimer disease during follow-up per standard deviation increase and per quartile in plasma clusterin levels

Clusterin ($\mu\text{g/ml}$)	Risk of incident Alzheimer disease					
	during total follow-up n=208		within three years n=76		after three years N=132	
	HR (95% CI) ¹	HR (95% CI) ²	HR (95% CI) ¹	HR (95% CI) ²	HR (95% CI) ¹	HR (95% CI) ²
Per SD	0.98 (0.84-1.15)	1.00 (0.85-1.17)	1.06 (0.82-1.37)	1.08 (0.84-1.42)	0.97 (0.80-1.17)	0.99 (0.81-1.20)
Per Quartile						
47.2 -99.5	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
99.6-115	1.07 (0.68-1.68)	1.07 (0.67-1.71)	0.82 (0.39-1.71)	0.85 (0.39-1.85)	1.24 (0.73-2.12)	1.23 (0.70-2.15)
116-132	1.07 (0.69-1.68)	1.12 (0.70-1.77)	1.30 (0.66-2.55)	1.34 (0.65-2.74)	0.97 (0.56-1.68)	1.00 (0.57-1.77)
133-198	0.90 (0.57-1.43)	0.92 (0.58-1.46)	1.01 (0.48-2.11)	1.04 (0.49-2.24)	0.91 (0.53-1.57)	0.96 (0.54-1.68)
P-trend	0.66	0.77	0.68	0.65	0.54	0.72

HR=hazard ratio, n=number of Alzheimer cases

¹ adjusted for age and sex² adjusted for age, sex, education, *APOE* ϵ 4-carriership, diabetes, smoking, coronary heart disease and hypertension**Table 5.** Odds of prevalent all-cause dementia and vascular dementia, and risk of incident all-cause dementia and vascular dementia during follow-up per SD increase in plasma clusterin levels

	Odds of prevalent dementia			Risk of incident dementia		
	Number of cases	OR (95% CI) ¹	OR (95% CI) ²	Number of cases	OR (95% CI) ¹	OR (95% CI) ²
All-cause dementia	77	1.45 (1.13-1.85)	1.51 (1.17-1.96)	237	0.95 (0.81-1.10)	0.96 (0.82-1.12)
Vascular dementia	9	1.47 (0.77-2.83)	1.50 (0.78-2.78)	20	0.84 (0.61-1.15)	0.82 (0.60-1.12)

OR=odds ratio, HR=hazard ratio

¹ adjusted for age and sex² adjusted for age, sex, education, *APOE* ϵ 4-carriership, diabetes, smoking, coronary heart disease, and hypertension

Table 6. Differences in cognitive test performance at baseline per standard deviation increase in plasma clusterin levels

		Random subcohort		Prevalent AD	
		N	Difference (95% CI)	N	Difference (95% CI)
MMSE (points)	Model 1	924	-0.004 (-0.128 to 0.120)	56	-1.34 (-2.54 to -0.13)
	Model 2	924	-0.005 (-0.126 to 0.116)	56	-1.36 (-2.70 to -0.02)
LDST (points)	Model 1	904	-0.010 (-0.437 to 0.417)	39	-0.95 (-2.85 to 0.94)
	Model 2	904	0.002 (-0.407 to 0.411)	39	-1.59 (-3.73 to 0.55)
WFT (points)	Model 1	916	0.22 (-0.13 to 0.58)	48	-0.48 (-1.69 to 0.73)
	Model 2	916	0.22 (-0.13 to 0.58)	48	-0.85 (-2.16 to 0.47)
Stroop trial 3 (seconds)	Model 1	898	0.38 (-0.89 to 1.65)	38	0.06 (-11.01; 11.13)
	Model 2	898	0.35 (-0.90 to 1.59)	38	1.72 (-11.56 to 14.99)

AD= Alzheimer disease, MMSE= Mini Mental State Examination, LDST= Letter Digit Substitution Task, WFT=Word Fluency Test

NB. For the MMSE, LDST and WFT more points mean a better test score, but for the Stroop test, more seconds indicate a longer time needed to complete the test, e.g. a worse test score.

Model 1: adjusted for age and sex

Model 2: adjusted for age and sex, education, *APOE* $\epsilon 4$ -carriership, diabetes, smoking, coronary heart disease and hypertension

DISCUSSION

In our population-based cohort study, plasma levels of clusterin were associated with the prevalence and severity of Alzheimer disease, but not with the development of incident Alzheimer disease during follow-up. Major strengths of our study were the population-based design and the long and virtually complete follow-up for incident dementia. Therefore, we were able not only to investigate the associations of plasma clusterin with the presence and severity of Alzheimer disease, but also to investigate whether plasma clusterin might be a preclinical marker of Alzheimer disease. However, magnetic resonance imaging was not routinely performed in the third survey; therefore, we were not able to investigate the relationship of plasma clusterin with brain or hippocampal atrophy. The relationship between plasma clusterin and progression of Alzheimer disease was also not investigated. We did explore the associations of clusterin with vascular dementia and all-cause dementia, which were similar to the associations with Alzheimer disease, suggesting that clusterin cannot be used to distinguish AD from vascular dementia. Other subtypes of dementia could not be investigated because of small numbers.

Our finding that plasma clusterin was associated with MMSE in patients with prevalent Alzheimer disease was similar to that of Thambisetty et al.,⁸ however, unlike their study, our patients with Alzheimer disease had significantly higher levels of plasma clusterin than controls. In addition, our data do not support the suggestion that clusterin is increased, possibly as an etiopathological event, before

the development of Alzheimer disease,⁸ but fits the hypothesis that the increased expression of clusterin in Alzheimer disease reflects a neuroprotective response.⁵ Several protective effects of clusterin on the brain that may play a role in Alzheimer disease have been described in in vitro or in vivo studies, including inhibition of amyloid formation²¹ through binding amyloid-beta or enhancing its clearance over the blood-brain barrier,²² clearance by endocytosis of amyloid-beta aggregates and cell debris to brain phagocytes, and inhibition of complement activation.⁵ The neurodegenerative changes that occur in Alzheimer disease may trigger an increased expression of clusterin.⁵ This is in line with our finding that plasma clusterin was associated with prevalent Alzheimer disease and severity of Alzheimer disease, but not with the risk of developing incident Alzheimer disease during follow-up. Clusterin was also associated with prevalent all-cause dementia and vascular dementia, supporting a reactive rather than a causative role of clusterin and suggesting that clusterin will not be useful in the differential diagnosis of Alzheimer disease versus other subtypes of dementia.

In conclusion, our data from the general population show that increased plasma clusterin levels are associated with prevalent Alzheimer disease and are higher in more severe cases of Alzheimer disease. However, increased levels of clusterin do not precede development of Alzheimer disease and therefore are not a potential early marker of subclinical disease.

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Retinal vascular abnormalities and
the risk of dementia

Chapter 4.1

Retinal vascular caliber and risk of dementia

Frank Jan de Jong,* Elisabeth M.C. Schrijvers,* M. Kamran Ikram,
Peter J. Koudstaal, Paulus T.V.M. de Jong, Albert Hofman, Johannes R. Vingerling,
Monique M.B. Breteler

*These authors contributed equally

ABSTRACT

Background Retinal vessels provide a unique opportunity to study both systemic and cerebrovascular disease. Smaller retinal arteriolar calibers are strongly related to hypertension, whereas larger retinal venular calibers are more related to inflammation, cerebral hypoperfusion, and cerebrovascular disease. Whether retinal vessel calibers are related to dementia remains unclear.

Methods We investigated whether retinal arteriolar and venular calibers are associated with risk of dementia, and its subtypes Alzheimer disease and vascular dementia, in the prospective population-based Rotterdam Study. Digitized retinal images were available in 5553 participants aged 55 years or over and dementia-free at baseline (1990-1993). Participants were re-examined in 1993-1994, 1997-1999 and 2002-2004 and were continuously monitored for development of dementia.

Results During a mean follow-up of 11.6 years, 655 participants developed dementia. Alzheimer disease was diagnosed in 519 and vascular dementia in 73 participants. Larger venular calibers were associated with an increased risk of dementia, in particular vascular dementia (age and sex adjusted hazard ratio per standard deviation increase: 1.31; 95% confidence interval: 1.06-1.64), but not Alzheimer disease. The association remained significant after adjustment for stroke and cardiovascular risk factors. Smaller arteriolar calibers were also associated with an increased risk of vascular dementia, yet only when adjusted for venular calibers.

Conclusions Retinal venular widening is associated with an increased risk of vascular dementia. Our findings are in line with previous observations in stroke and cerebral small vessel disease and suggest that the association between larger retinal venular calibers and dementia may reflect cerebral hypoperfusion and subsequent ischemia.

INTRODUCTION

Dementia is a leading cause of morbidity in the elderly, yet the exact causes remain unclear and treatment options are limited. Cerebrovascular disease is thought to play a role in the pathogenesis of dementia and its major subtypes Alzheimer disease and vascular dementia.¹ The cerebral microcirculation is, however, difficult to assess and most non-invasive indicators of vascular pathology relate to vessel beds outside the brain. Retinal vessels provide a unique insight into the brain's microvasculature, because embryological, anatomical and physiological characteristics are similar to the cerebral circulation and the retina is easy to visualize non-invasively.^{2,3} Moreover, pathological changes in the retinal microcirculation have been shown in patients with cerebrovascular disease, suggesting that retinal vessels may reflect concomitant cerebral microangiopathy.^{4,5}

During the late 1990s, a semi-automated system became available to reliably quantify retinal arteriolar and venular calibers.⁶ Several studies have shown that smaller arteriolar calibers were strongly related to higher blood pressure,⁷⁻⁹ whereas larger venular calibers were consistently associated with higher levels of inflammation markers, cholesterol, and both sub-clinical and clinical atherosclerosis.^{7,8,10-12} Furthermore, larger venular calibers were associated with an increased risk of stroke and progression of cerebral small vessel disease.¹³⁻¹⁷ We studied the associations between retinal arteriolar and venular calibers, and risk of dementia and its major subtypes Alzheimer disease and vascular dementia, using data from a population-based cohort study.

METHODS

Study population

The study was conducted as part of the Rotterdam Study, a large population-based prospective cohort study among all inhabitants aged 55 years and over of Ommoord, a district of Rotterdam, the Netherlands.¹⁸ Of 10,274 eligible subjects, 7983 (78%) participated in the baseline examinations between 1990-1993. The medical ethics committee at Erasmus University of Rotterdam approved the study and written informed consent was obtained from all participants.

Since eye examinations became operational a few months after the baseline examinations had started, a smaller number (n = 6780) participated in the ophthalmic part of the study. Due to technical reasons (mostly absence of technicians) fundus transparencies were not available for 344 participants. Fundus transparencies were available in 6436 participants, and of these, 6432 participants were screened for dementia, of

whom 213 were diagnosed with dementia at baseline. Fundus transparencies were ungradable in 666 of the 6219 participants who were free from dementia and underwent the eye examination at baseline. The cohort at risk of dementia with gradable retinal vessel measurements at baseline thus comprised 5553 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. Follow-up for dementia was virtually complete until January 1, 2007.

Dementia diagnoses

Participants were screened for dementia with a three-step procedure, which was similar at baseline and follow-up examinations.¹⁹ First, participants were cognitively screened with the Mini-Mental State Examination (MMSE)²⁰ and the Geriatric Mental State schedule (GMS)²¹ organic level. Second, if participants scored below 26 on the MMSE or above 0 on the GMS organic level, the Cambridge Examination of Mental Disorders in the Elderly (CAMDEX)²² was administered, and an informant was interviewed. Finally, participants suspected of having dementia were further examined by a neurologist, a neuropsychologist and, if possible, had magnetic resonance imaging of the brain. In addition, continuous monitoring of the cohort for incident dementia cases took place through direct linkage between the study database and computerized medical records from general practitioners and through surveillance of Regional Institute for Outpatient Mental Health Care reports. The diagnosis of dementia and subtype of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III-R)²³, Alzheimer disease (NINCDS-ADRDA)²⁴ and vascular dementia (NINDS-AIREN criteria).²⁵ As proposed in the latter criteria we recognized a subgroup of Alzheimer disease with cerebrovascular disease. Diagnoses were made on all available information by an expert panel including the neurologist, neuropsychologist and research physician.

Grading of retinal vascular calibers

At the baseline ophthalmic examination, fundus colour transparencies were taken centered on the optic disk (20° field, Topcon Optical Company, Tokyo, Japan) after pharmacological mydriasis and were digitized with a high-resolution scanner (Nikon LS-4000, Nikon Corporation, Japan). For each participant the digitized image with the best quality of either eye was analyzed with the Retinal Vessel Measurement System (Retinal Analysis, Optimate, WI; Department of Ophthalmology & Visual Science, University of Wisconsin-Madison, USA).⁶

The rationale and procedures to measure and summarize retinal vascular calibers have been described.^{6,7} Summary measures for arteriolar and venular calibers were based on improved Parr-Hubbard formulas and were corrected for magnification changes due to refractive errors of the eye. Four trained graders performed the assessments, masked to the clinical characteristics of the participants. A random sub-sample of 40 transparencies was used to monitor quality of the data at regular intervals. Pearson's correlation coefficients for intergrader agreement were 0.67-0.80 (arteriolar calibers) and 0.91-0.94 (venular calibers). For intragrader agreement these figures were 0.69-0.88 (arteriolar calibers) and 0.90-0.95 (venular calibers).

Other variables

Smoking habits (categorized as current, former and never smoking) and use of anti-hypertensive medication were assessed during the baseline interview. Blood pressure was measured twice with a random zero sphygmomanometer at the brachial artery with the subject in sitting position, and the measurements were averaged. Non-fasting serum total cholesterol concentrations were determined by an automated enzymatic procedure. Serum levels of high-sensitive C-reactive protein (CRP) were determined by the Rate Near Infrared Particle Immunoassay method (Image[®] high-sensitive CRP, Beckman Coulter, USA). Diabetes mellitus was defined as a self-reported history of diabetes or a random non-fasting or post-load serum glucose level ≥ 11.1 mmol/l. History of stroke at baseline was assessed during the baseline interview and verified by reviewing medical records. After enrollment, participants were continuously monitored for incident stroke through automated linkage of the study database with files from general practitioners and the municipality. Additional information was obtained from hospital records. Coronary heart disease was defined as a previous myocardial infarction, PTCA or coronary bypass. Apolipoprotein E (*APOE*) genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis.²⁶ *APOE* $\epsilon 4$ -carriership was defined as the presence of at least one *APOE* $\epsilon 4$ -allele.

Statistical analysis

Analysis of covariance (ANCOVA), adjusted for age and sex, was used to compare baseline characteristics of participants with and without gradable fundus transparencies. Associations between baseline retinal vascular calibers and incident dementia, Alzheimer disease (with or without cerebrovascular disease), and vascular dementia were assessed with Cox proportional hazards models. Participants were followed until diagnosis of dementia, death, or end of study, whichever came first. Hazard

ratios (HR) were adjusted for age and sex. Retinal arteriolar and venular calibers were first entered in quintiles of their distribution to check whether their relations with dementia were nonlinear. Since associations did not obviously deviate from linearity, all analyses were subsequently performed entering retinal vascular characteristics as a linear term in the model. HRs were expressed per standard deviation (SD) difference in retinal vascular calibers to allow comparison of strength of associations across the different vascular characteristics. We tested the proportional hazard assumption by including the interactions of the vessel characteristics with time as covariate in the model. Interaction terms of both arteriolar and venular calibers with follow-up time were all non-significant, indicating that the associations between vascular calibers and dementia did not differ according to length of follow-up. To control for the confounding effect of the other vessel, we subsequently entered both calibers simultaneously in the model.²⁷⁻²⁹ All analyses were additionally adjusted for the abovementioned cardiovascular risk factors. Stroke before the end of follow-up was included in the model as a time-varying covariate. Because the *APOE* ϵ 4-allele is an important risk factor for Alzheimer disease,³⁰ and may modulate the effects of vascular disease on the brain,³¹ we also performed the analyses within strata of *APOE* genotype (carriers vs. non-carriers of the ϵ 4-allele). All analyses were performed using SPSS statistical software version 15 (SPSS Inc., Chicago, Illinois).

RESULTS

Baseline characteristics of the study population and a comparison between participants with gradable and ungradable fundus transparencies are shown in table 1. Adjusted mean differences show that those with ungradable fundus transparencies were significantly older, more often institutionalized and had higher blood pressures. There were no significant differences in other risk factors. The mean summated arteriolar caliber was 147.0 μ m (SD: 14.4 μ m; range 92.2-235.7 μ m), and the mean summated venular caliber 222.2 μ m (SD: 20.8 μ m; range 135.1-313.6 μ m). After a follow-up of 64,549 person-years (mean: 11.6 years (SD: 4.4)), 655 participants had developed dementia, of whom 519 were diagnosed with Alzheimer disease (472 without and 47 with cerebrovascular disease) and 73 with vascular dementia. The remaining 63 cases were ascribed to other subtypes (including dementia in Parkinson's disease, multi system atrophy and Lewy body dementia). Table 2 shows the association of retinal arteriolar and venular calibers with risk of dementia. When analyses were adjusted only for age and sex we found no association of arteriolar calibers with risk of dementia, whereas larger venular calibers were associated with a higher risk of dementia. Analyses according to dementia subtype showed that the associa-

Table 1. Baseline characteristics of the study population

	Gradable* N=5553	Ungradable N=666	Adjusted differences† (95% CI)
Age (years)	67.7 ± 8.0	74.5 ± 9.8	6.7 (6.1;7.4)
Women (%)	58.6	60.8	-0.6 (-4.7;3.4)
Institutionalized (%)	2.8	15.2	6.6 (5.1;8.1)
Total cholesterol (mmol/L)	6.65 ± 1.22	6.59 ± 1.28	0.04 (-0.06;0.14)
High-sensitive C-reactive protein (mg/L)	3.15 ± 6.08	4.10 ± 11.0	0.48 (-0.10;1.05)
Current smoking (%)	23.7	19.6	0.6 (-2.9;4.1)
Diabetes (%)	9.7	14.5	1.4 (-1.1;3.9)
Stroke at baseline (%)	2.2	4.5	0.9 (-0.3;2.2)
Coronary heart disease (%)	8.2	9.2	-0.7 (-3.0;1.5)
Systolic blood pressure (mmHg)	138.4 ± 22.0	145.3 ± 23.8	2.1 (0.3;4.0)
Diastolic blood pressure (mmHg)	73.7 ± 11.3	74.2 ± 12.4	1.3 (0.4;2.3)
Use of antihypertensive medication (%)	30.5	38.7	-0.3 (-4.4;3.7)

Data are presented as unadjusted mean ± standard deviation or percentages

* Participants with a gradable fundus transparency of at least one eye

† age and/or sex adjusted mean differences between participants with a gradable fundus transparency and those with ungradable fundus transparency

Table 2. Risk of dementia, Alzheimer disease and vascular dementia according to retinal vascular calibers

	Dementia	Alzheimer disease		Vascular dementia
	n=655 HR (95% CI)	without CVD n=472 HR (95% CI)	with CVD n=47 HR (95% CI)	n=73 HR (95% CI)
Arteriolar caliber				
Per SD (14.4 µm) decrease, model 1	0.99 (0.91-1.07)	0.98 (0.89-1.07)	1.14 (0.85-1.52)	1.06 (0.84-1.33)
Per SD (14.4 µm) decrease, model 2	1.06 (0.96-1.16)	1.02 (0.91-1.14)	1.25 (0.88-1.79)	1.40 (1.05-1.86)
Per SD (14.4 µm) decrease, model 3	1.05 (0.96-1.16)	1.02 (0.91-1.14)	1.27 (0.89-1.82)	1.33 (0.99-1.78)
Venular caliber				
Per SD (20.8 µm) increase, model 1	1.09 (1.01-1.18)	1.06 (0.97-1.16)	1.03 (0.77-1.37)	1.31 (1.06-1.64)
Per SD (20.8 µm) increase, model 2	1.13 (1.02-1.24)	1.07 (0.96-1.20)	1.17 (0.82-1.67)	1.59 (1.21-2.09)
Per SD (20.8 µm) increase, model 3	1.11 (1.00-1.22)	1.06 (0.95-1.19)	1.16 (0.82-1.64)	1.44 (1.10-1.89)

CVD= cerebrovascular disease, n=number of cases

Model 1: adjusted for age and sex

Model 2: 1 + the caliber of the other vessel

Model 3: 2 + systolic blood pressure, antihypertensive medication, serum total cholesterol, serum C-reactive protein, smoking, diabetes mellitus, coronary heart disease and stroke

tion of larger venular calibers with an increased risk of dementia was driven by the association with vascular dementia. For every SD increase in venular caliber, risk of vascular dementia increased significantly by 31%. This association became even more pronounced after additional correction for arteriolar caliber (HR per SD increase in venular caliber 1.59, 95% confidence interval (CI) 1.21-2.09). Further adjustments for

stroke and cardiovascular risk factors only slightly attenuated the association (HR per SD increase in venular caliber 1.44, 95 % CI 1.10-1.89). Venular calibers were not related to the risk of Alzheimer disease without cerebrovascular disease, regardless of correction for arteriolar caliber (HR per SD increase in venular caliber after correction for arteriolar caliber 1.07, 95% CI 0.96-1.20). The risk of Alzheimer disease with cerebrovascular disease increased with 17% per SD increase in venular caliber after correction for arteriolar caliber, although this was non-significant.

When analyzed separately, arteriolar calibers were neither related to Alzheimer disease nor to vascular dementia. Yet, after correction for venular caliber, we observed a significant association of arteriolar calibers with vascular dementia, but not with Alzheimer disease. The association with vascular dementia became borderline significant after adjustment for stroke and cardiovascular risk factors. The risk of Alzheimer disease with cerebrovascular disease non-significantly increased with 25% per SD decrease in arteriolar caliber.

For both arteriolar and venular calibers the association with dementia was similar for participants with or without at least one *APOE* $\epsilon 4$ -allele.

DISCUSSION

In our prospective study, we have investigated the association of retinal vascular calibers with the risk of developing dementia. One previous study investigated the association of retinal vascular calibers with presence of dementia, but found no association of retinal vascular calibers and cognitive function or dementia.³² This study was, however, cross-sectional. Only a few more studies have investigated the relation between retinal vascular calibers and cognitive function, reporting either no association or an association of larger venular calibers with impaired cognitive function.³³⁻³⁵ In these studies, the most often reported retinal vessel characteristic was the ratio of arteriolar-to-venular caliber, which does not provide information on the individual contribution of the arteriolar and venular calibers.

Our results are in agreement with previous findings showing that larger venular calibers are associated with progression of cerebral small vessel disease and stroke,¹³⁻¹⁷ both major risk factors for vascular dementia. The observation that the association was less strong and non-significant for Alzheimer disease with cerebrovascular disease, and absent for Alzheimer disease without cerebrovascular disease, is also in concordance with these findings.

Larger retinal venular calibers may be related to vascular dementia in several ways. First, they may reflect exposure to clinical stroke or other vascular risk factors, including atherosclerosis, inflammation, diabetes mellitus and smoking. Since adjust-

ing for these factors did not change results, other mechanisms should be considered. Second, larger retinal venular calibers have been hypothesized to be a general marker of retinal ischemia and by proxy of cerebral ischemia.¹⁵ Retinal venular dilatation is observed in the early stages of diabetic and venous stasis retinopathy, both of which are characterized by retinal ischemia.^{12,36} In turn, retinal ischemia has been related to lower cerebral blood flow.¹² In line with these observations, larger retinal venular calibers were found to be associated with several indicators of lower cerebral oxygen supply. We reported lower arteriolar oxygen saturation to be associated with larger retinal venular calibers in particular in the presence of lower cerebral blood flow.³⁷ In addition, larger venular calibers were found to be associated with severe extracranial carotid artery disease in patients with acute ischemic stroke. This association was confined to retinal venular widening ipsilateral to the carotid artery stenosis.³⁸ Altogether, this suggests that cerebral hypoperfusion may explain the relation between retinal venular widening and increased dementia risk.

Venous stasis may cause cerebral hypoperfusion and ischemia in particular in the periventricular region of the brain through diminished clearance of cellular metabolites and as such contribute to the development of white matter lesions and ultimately dementia.³⁹ Because brain imaging was not performed routinely in all participants, we were not able to investigate whether white matter lesions account for the association we found between larger venular calibers and dementia-risk.

Retinal arteriolar narrowing was also associated with vascular dementia, albeit to a lesser extent. Smaller arteriolar calibers are strongly related to hypertension,^{7,8} which is one of the strongest risk factors for both stroke and vascular dementia. An association of smaller arteriolar calibers with vascular dementia was therefore expected. Yet, smaller arteriolar calibers were related to an increased risk of vascular dementia only after adjustment for venular calibers. The absence of an association in the overall analysis may well be the result of opposing effects of arteriolar narrowing caused by hypertension on the one hand and arteriolar widening caused by endothelial dysfunction and ischemia on the other hand. Due to increased arterial stiffness as a result of vasoconstriction, intimal thickening, medial hyperplasia, hyalinisation and sclerosis at higher age, widening of retinal arterioles may be less pronounced than widening of retinal venules in conditions reflecting ischemia.¹⁵ In addition, arteriolar and venular calibers are correlated and persons with larger venular calibers in general also have larger arteriolar calibers. The effect of smaller arteriolar calibers on dementia-risk is therefore masked by a confounding effect of larger venular calibers in model 1.²⁷

Important strengths of our study are the population-based design and the long follow-up, which was virtually complete with regard to the dementia diagnosis. Other advantages of our study are the detailed assessment of retinal vascular

calibers on 20° stereoscopic transparencies obtained after pharmacological mydriasis and the adjustment for refractive errors of the eye. This enabled us to estimate the intra-luminal arteriolar and venular calibers in more detail, where others reported uncorrected calibers in pictures with smaller magnification.⁶

Some further methodological issues should be discussed. Participants who did not visit the study center to undergo the ophthalmic examination and participants with an ungradable fundus transparency were on average older and more often institutionalized. Given the long duration and the completeness of follow-up in our study, distortion of the reported associations by selection bias is unlikely. Limitations related to the semi-automated system assessing the retinal vascular calibers have been described.⁶ Because assessment of retinal calibers was unrelated to clinical characteristics of the participants, these limitations most likely led to an underestimation of our effects due to random misclassification.

Future studies are needed to confirm our findings of an association between larger retinal venular caliber and vascular dementia-risk. Imaging techniques as CT perfusion and MR angiography studies should be added in order to determine whether cerebral hypoperfusion indeed provides the mechanism underlying the association of venular widening with an increased risk of vascular dementia.

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Chapter 4.2

Retinopathy and risk of dementia

Elisabeth M.C. Schrijvers, Gabriëlle H.S. Buitendijk, M. Kamran Ikram,
Peter J. Koudstaal, Albert Hofman, Johannes R. Vingerling, Monique M.B. Breteler

ABSTRACT

Background Because of similarities between the retinal and the cerebral circulation, retinal microvascular signs can be used as a marker of cerebral microvascular disease. Retinopathy has been associated with dementia cross-sectionally, but data from prospective cohorts are lacking.

Methods We investigated the associations between retinopathy and dementia and its subtypes Alzheimer disease and vascular dementia both cross-sectionally and prospectively in the Rotterdam Study, a large population-based cohort study.

Digitized retinal images were available in 195 participants with prevalent dementia and 6078 participants without dementia at baseline (1990-1993). Participants were re-examined in 1993-1994, 1997-1999 and 2002-2004 and were continuously monitored for development of dementia until January 1, 2007. Retinopathy was graded on fundus photographs and was defined as the presence of one or more dot/blot hemorrhages, microaneurysms, cotton wool spots or evidence of laser treatment for retinopathy.

Results Retinopathy was associated with prevalent dementia (age and sex adjusted odds ratio 2.04, 95% confidence interval [CI] 1.34-3.09). Results were similar for Alzheimer disease and vascular dementia. During a mean follow-up of 11.4 years, 735 participants developed incident dementia, of whom 583 were diagnosed with Alzheimer disease and 80 with vascular dementia. There was no association of retinopathy at baseline with the risk of incident dementia during follow-up (age and sex adjusted hazard ratio 1.15, 95% CI 0.88-1.48) or the risk of incident Alzheimer disease or vascular dementia.

Conclusions Retinopathy is more prevalent in persons with dementia, but is not associated with an increased risk of dementia over time.

INTRODUCTION

Dementia is a major cause of morbidity and mortality in elderly people. Many factors contribute to the development of dementia, and although the exact causes are still unclear, cerebrovascular disease is thought to be an important risk factor.¹ To study the role of cerebral microvascular disease in the pathogenesis of dementia, there is much interest in retinal microvascular signs, because embryological, anatomical and physiological characteristics of the retinal vasculature are similar to the cerebral circulation and the retina is easy to visualize non-invasively.^{2,3} We have previously shown an association of larger retinal venular caliber and smaller arteriolar caliber with the risk of developing vascular dementia.⁴ Another interesting retinal microvascular sign that has been associated with cognition and dementia is retinopathy.⁵⁻⁹ In prospective MRI studies, retinopathy has also been associated with the development of silent brain infarcts¹⁰ and ventricular enlargement¹¹ over a 10 year period; features that also have been associated with dementia¹² or cognition.¹³ Currently, studies investigating the association between retinopathy and the development of dementia in a prospective setting are lacking.

In this study, we investigated the associations of retinopathy with both prevalent and incident dementia and its subtypes Alzheimer disease and vascular dementia in the Rotterdam Study, a large population-based cohort study.

METHODS

Study population

The Rotterdam Study is a prospective population-based cohort study that is conducted among all inhabitants aged 55 years and over of Ommoord, a district of Rotterdam, the Netherlands.¹⁴ Of 10,274 eligible subjects, 7983 (78%) participated in the baseline examinations between 1990-1993. The medical ethics committee at Erasmus University of Rotterdam approved the study and written informed consent was obtained from all participants. Since eye examinations became operational a few months after the baseline examinations had started, a smaller number (n=6787) participated in the ophthalmic part of the study. Due to technical reasons (mostly absence of technicians) fundus photographs were not available for 352 participants. Fundus photographs were available in 6435 participants, and of these, 3 were ungradable. We excluded participants with end-stage age-related macular degeneration (n=106), retinal arterial or vein occlusions (n=51), or if they were not screened for dementia (n=2).

This resulted in a study population of 6273 participants, of whom 195 were diagnosed with dementia at baseline. The cohort at risk of dementia at baseline thus comprised 6078 participants. Follow-up examinations were conducted in 1993 to 1994, 1997 to 1999 and 2002 to 2004. In addition, through linkage with records of general practitioners, the total cohort was continuously monitored for morbidity and mortality. Follow-up for dementia was virtually complete until January 1, 2007.

Dementia diagnoses

Participants were screened for dementia with a three-step procedure, which was similar at baseline and follow-up examinations.¹⁵ Two brief tests of cognition (Mini-Mental State Examination (MMSE)¹⁶ and Geriatric Mental State schedule (GMS)¹⁷ organic level) were used to screen all participants. Screen-positives (MMSE score <26 or GMS organic level >0) underwent the Cambridge examination for mental disorders of the elderly (Camdex).¹⁸ Persons 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 digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.¹⁵ The diagnoses of dementia and its subtypes were made in accordance with internationally accepted criteria for dementia (DSM-III-R),¹⁹ Alzheimer disease (NINCDS-ADRDA),²⁰ and vascular dementia (NINDS-AIREN)²¹ by a panel of a neurologist, neuropsychologist and research physician.

Retinopathy

Ophthalmic history was taken from the study participants before they underwent ophthalmic examination and fundus photography, covering a 35° field centered on the macula after pharmacological mydriasis at each visit of both eyes (Topcon TRV-50VT fundus camera, Topcon Optical Co, Tokyo, Japan). Fundus photographs were checked for quality and the presence of age-related macular degeneration, using the International Classification and Grading System. Study participants with ungradable photographs, end-stage age-related macular degeneration and retinal arterial or vein occlusions were excluded from further analyses. The presence of cotton wool exudates and the presence and number of dot/blot hemorrhages were graded, without differentiation between microaneurysms and hemorrhages. Additionally the presence of laser coagulation scars were graded and the indication for laser therapy was categorized into either retinopathy or other diseases (most often retinal vein occlusion), using available clinical data. Retinopathy was defined as the presence of

one or more dot/blot hemorrhages, microaneurysms, cotton wool spots or evidence of laser treatment for retinopathy.

Other variables

Smoking habits and use of anti-hypertensive medication were assessed during the baseline home interview. Blood pressure was measured twice with a random zero sphygmomanometer at the brachial artery with the subject in sitting position, and the measurements were averaged. Hypertension was defined as a blood pressure $\geq 140/90$ or use of anti-hypertensive medication, prescribed for the indication of hypertension. Non-fasting serum total cholesterol concentrations were determined by an automated enzymatic procedure. Serum levels of high-sensitive C-reactive protein (CRP) were determined by the Rate Near Infrared Particle Immunoassay method (Image® high-sensitive CRP, Beckman Coulter, USA). Diabetes mellitus was defined as a self-reported history of diabetes or a random non-fasting or post-load serum glucose level ≥ 11.1 mmol/l. History of stroke at baseline was assessed during the baseline interview and verified by reviewing medical records. After enrollment, participants were continuously monitored for incident stroke through automated linkage of the study database with files from general practitioners and the municipality. Additional information was obtained from hospital records. Coronary heart disease was defined as a previous myocardial infarction, percutaneous transluminal coronary angioplasty or coronary bypass. Apolipoprotein E (*APOE*) genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis.²² *APOE* $\epsilon 4$ -carriership was defined as the presence of at least one *APOE* $\epsilon 4$ -allele. Missing values in covariates (6% or less) were imputed based on age and sex for continuous variables and as the mean for dichotomous variables.

Statistical Analyses

First, we investigated the cross-sectional associations between retinopathy and prevalent dementia, Alzheimer disease and vascular dementia. We used logistic regression analyses adjusted for age and sex (model 1), with additional adjustments for stroke (model 2) and for systolic blood pressure, use of antihypertensive medication, educational level, *APOE* $\epsilon 4$ -carriership, current cigarette smoking, diabetes, total cholesterol, CRP and coronary heart disease.

Next, we used Cox proportional hazard models to investigate the associations between retinopathy at baseline and incident dementia, Alzheimer disease or vascular dementia during follow-up. Adjustments were made for the abovementioned

covariates. Stroke before the end of follow-up was included in the model as a time-varying covariate. We tested the proportional hazard assumption by including the interaction of retinopathy with time as covariate in the models. Interaction terms of retinopathy with follow-up time were non-significant, indicating that the associations of retinopathy with dementia, Alzheimer disease and vascular dementia did not differ according to length of follow-up.

We repeated both the cross-sectional and the prospective analyses in strata of hypertension and diabetes and tested for interactions of diabetes or hypertension with retinopathy by adding an interaction term of diabetes and retinopathy, and an interaction term of hypertension and retinopathy to model 1.

RESULTS

Baseline characteristics of prevalent dementia cases and the cohort at risk for incident dementia are shown in Table 1. Of the 195 prevalent dementia cases, 149 were diagnosed with Alzheimer disease, 29 with vascular dementia, and 17 with other subtypes of dementia. Associations of retinopathy with prevalent dementia are shown in Table 2. Retinopathy was significantly associated with dementia (OR 2.04, 95% CI 1.34-3.09, adjusted for age and sex). This association remained the same

Table 1. Baseline characteristics of the study population

	Cohort at risk N=6078	Prevalent dementia N=195
Age (years)	68.3 ± 8.4	81.6 ± 7.5
Female (%)	59	69
Only primary education (%)	36	73
Carrier of APOE e4-allele (%)	28	43
Current cigarette smoking (%)	21	12
Systolic blood pressure (mmHg)	139 ± 22	144 ± 24
Diastolic blood pressure (mmHg)	74 ± 11	73 ± 14
Use of anti hypertensive medication (%)	31	41
Hypertension (>=140/90)	55	64
CRP (mg/L)	3.23 ± 6.71	5.48 ± 11.1
Total cholesterol (mmol/L)	6.65 ± 1.22	6.27 ± 1.24
Diabetes (%)	10	23
Stroke (%)	2	15
Coronary heart disease (%)	8	12
Retinopathy (%)	7	18

Data are presented as mean ± standard deviation or percentages

Table 2. Odds ratios of retinopathy and prevalent dementia

			Model 1	Model 2	Model 3
		Cases/N	OR (95% CI)	OR (95% CI)	OR (95% CI)
All dementia	No retinopathy	160/5800	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Retinopathy	34/473	2.04 (1.34-3.09)	1.90 (1.25-2.91)	1.92 (1.24-2.98)
Alzheimer disease	No retinopathy	124/5764	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Retinopathy	25/463	1.80 (1.11-2.91)	1.81 (1.12-2.94)	1.89 (1.15-3.10)
Vascular dementia	No retinopathy	22/5662	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Retinopathy	7/445	3.01 (1.26-7.21)	2.12 (0.80-5.62)	2.00 (0.71-5.63)

Model 1: adjusted for age and sex

Model 2: + stroke

Model 3: + systolic blood pressure, use of antihypertensive medication, education, *APOE e4*-carriership, current cigarette smoking, diabetes, total cholesterol, crp and coronary heart disease

after adjustment for stroke and other possible confounders. For Alzheimer disease the associations were similar. The association of retinopathy with vascular dementia seemed stronger than the associations with all-cause dementia and Alzheimer disease in the first model (OR 3.01, 95% CI 1.26-7.21), but after adjustment for stroke the effect size became similar, although non-significant, to the effect sizes observed for overall dementia and Alzheimer disease (OR 2.12, 95% 0.80-5.62). Analyses stratified for hypertension or diabetes showed no differences between strata (p-values of interaction terms for dementia and Alzheimer disease ≥ 0.36 , data not shown). For vascular dementia, there was no interaction of diabetes with retinopathy (p=0.48), but the association with retinopathy seemed stronger in persons without hypertension (p=0.059). This difference disappeared after adjustment for stroke (p=0.31).

In the cohort at risk, 735 participants had developed dementia after a mean follow-up of 11.4 years (max 17.3 years), of whom 583 were diagnosed with Alzheimer disease, 80 with vascular dementia and 72 with other types of dementia. There was no association of retinopathy at baseline with incident dementia, Alzheimer disease or vascular dementia. (Table 3) In strata of hypertension and diabetes, there was also no association of retinopathy with incident dementia, Alzheimer disease or vascular dementia. (Data not shown) Interactions terms of diabetes and hypertension with retinopathy were all non-significant (p-values>0.12).

DISCUSSION

Our data suggest that retinopathy is more prevalent in persons with dementia, but does not precede the development of incident dementia over time. Although pres-

Table 3. Hazard ratios of retinopathy and risk of incident dementia during follow-up

			Model 1	Model 2	Model 3
		Cases/N	HR (95% CI)	HR (95% CI)	HR (95% CI)
All dementia	No retinopathy	672/5640	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Retinopathy	63/438	1.15 (0.88-1.48)	1.13 (0.88-1.47)	1.15 (0.89-1.50)
Alzheimer disease	No retinopathy	534/5640	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Retinopathy	49/438	1.12 (0.83-1.50)	1.12 (0.83-1.50)	1.15 (0.86-1.55)
Vascular dementia	No retinopathy	74/5640	1.00 (ref)	1.00 (ref)	1.00 (ref)
	Retinopathy	6/438	0.97 (0.42-2.23)	0.92 (0.40-2.12)	0.90 (0.39-2.11)

Model 1: adjusted for age and sex

Model 2: + stroke

Model 3: + systolic blood pressure, use of antihypertensive medication, education, *APOE e4*-carriership, current cigarette smoking, diabetes, total cholesterol, crp and coronary heart disease

ence of retinopathy might be used as general marker for microvascular pathology, it will not be useful as an early marker or risk predictor for dementia.

Strengths of our study include the population-based design, the large number of participants and the virtually complete follow-up for incident dementia. Furthermore, we were able to investigate the associations of retinopathy with both prevalent and incident dementia, and its major subtypes Alzheimer disease and vascular dementia. Some further methodological issues should be discussed. Only the central field (ETDRS Field 2) was taken and used for grading of retinopathy and we did not perform a fluorescein angiography, which is a more invasive but more sensitive method to assess retinopathy. Any possible underestimation of retinopathy due to these methods will however be unrelated to the clinical characteristics of our participants and will therefore be random.

Our results are in line with cross-sectional studies that found an association between retinopathy and dementia^{5,8} or lower performance on cognitive tests^{7,9}, although there are some differences. In the Cardiovascular Health Study retinopathy was associated with dementia only in persons with hypertension or without diabetes⁵, and in the Blue Mountain Eye Study there was an association between retinopathy and a low score on the MMSE (<23) only in persons with hypertension. We found an association of retinopathy and prevalent dementia in the whole population and found no differences between persons with or without hypertension and diabetes. In the AGES-Reykjavik Study retinopathy was associated with vascular dementia, but not with all-cause dementia or Alzheimer disease.⁸ Although this is in line with our findings regarding the relation between retinal vascular caliber and incident dementia, where we also found an association with vascular dementia only,⁴ we found no differences with retinopathy between subtypes of dementia. Interestingly,

in the ARIC cohort, the investigators found an association of retinopathy with worse performance on three cognitive tests measuring memory, psychomotor speed and executive functioning in cross-sectional analyses,⁹ while prospectively they only found an association with psychomotor speed and executive functioning, and not with memory.⁶ In their discussion, the authors hypothesized that retinal vascular changes are related to the cognitive impairment which is specifically microvascular in origin, rather than memory, which is more related to Alzheimer disease.⁶ Our current study, however, does not support this hypothesis.

It is of interest that we did not find an association of retinopathy with incident dementia or vascular dementia. Because retinopathy is considered to reflect more severe retinal microvascular disease and a breakdown of the blood-retinal barrier^{3,5} and we had previously found an association of retinal vascular caliber with incident vascular dementia,⁴ we had expected to find an association of retinopathy with incident overall or vascular dementia. One possible explanation for us not finding such a relation may be that retinopathy is a dichotomous exposure and, compared to the continuous measures of retinal vascular caliber, lacks power to find an association with vascular dementia, for which numbers of cases are limited. The effect size, however, does not support any association with incident vascular dementia. Next, a competing risk effect of mortality could lead to an underestimation of the effect, although it is unlikely that this would completely obscure the effect. Furthermore, although retinal vascular caliber and retinopathy are both considered retinal microvascular signs, they can reflect different pathologies. Whereas our previous association between retinal venular caliber and the risk of incident vascular dementia might reflect cerebral hypoperfusion,⁴ retinopathy might be more a reflection of arteriolar damage from hypertension and diabetes.³ Finally, it is unlikely that retinal microvascular abnormalities are a causal factor in the development of dementia or cognitive decline. They are more likely a marker of underlying microvascular disease, which might contribute to the development of dementia or cognitive decline. Perhaps, because retinopathy reflects a more severe stage of the microvascular abnormalities it is actually not implausible that we only see this endstage of vascular pathology occurring at about the same time as dementia, but not before the clinical symptoms of dementia start developing. This would also explain why we find no differences between prevalent all-cause dementia, Alzheimer disease and vascular dementia, because vascular pathology often coexists with Alzheimer disease pathology.¹

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5

Endocrine factors and the risk of dementia

Chapter 5.1

Insulin metabolism and the risk of Alzheimer disease

Elisabeth M.C. Schrijvers, Jacqueline C.M. Witteman, Eric J.G. Sijbrands,
Albert Hofman, Peter J. Koudstaal, Monique M.B. Breteler,

ABSTRACT

Background Diabetes mellitus has been associated with an increased risk of Alzheimer disease, but how it exerts its effect remains controversial. Possible pathophysiological mechanisms are glucose toxicity and a direct effect of insulin on amyloid metabolism. Most studies had short follow-up and longer term effects of diabetes on Alzheimer disease risk are unknown. We investigated whether fasting glucose and insulin levels, and insulin resistance are associated with the risk of Alzheimer disease and if this risk is constant over time.

Methods The study was based on 3139 participants of the Rotterdam Study, a population-based cohort study. All subjects were free from dementia, without a history of diabetes, and had fasting levels of glucose and insulin measured at baseline. Insulin resistance was estimated with the homeostasis model assessment. We investigated how fasting glucose, insulin, and insulin resistance are related to the risk of Alzheimer disease in three different strata according to time-to-event, using Cox proportional hazards models.

Results During follow-up 211 participants developed Alzheimer disease, 71 of them within three years of baseline. Levels of insulin and insulin resistance were associated with a higher risk of Alzheimer disease within three years of baseline. After three years the risk was no longer increased. Glucose was not associated with a higher risk of Alzheimer disease. There was no interaction of *APOE* ϵ 4-carriership and insulin metabolism on the risk of Alzheimer disease.

Conclusions Our findings suggest that insulin metabolism influences the clinical manifestation of Alzheimer disease only within three years.

INTRODUCTION

Diabetes and Alzheimer disease are both common diseases in the elderly. Although many studies have suggested that patients with diabetes have a higher risk of developing Alzheimer disease,^{1,2} the results are conflicting and the real relationship between diabetes and Alzheimer disease remains controversial.

Three characteristics related to type 2 diabetes that might underlie the effect of diabetes on the brain in the development of Alzheimer disease are insulin resistance, hyperinsulinemia, and hyperglycemia.³⁻⁵ These mechanisms are thought to act on different pathways that are important in the pathophysiology of Alzheimer disease, either indirectly, through inflammation or the development of vascular disease, or directly, through effects on amyloid and tau metabolism and the formation of advanced glycation end products.^{1,5-7}

We previously reported from the Rotterdam study, a population based cohort study, an association between diabetes and a higher risk of Alzheimer disease.² This study was based on the initial cohort that started in 1990 and had only a short follow-up of on average 2.1 years.^{1,2} In 1997, at the third survey of the Rotterdam study, fasting blood samples were drawn. Using this survey as baseline for our current study, we now have levels of fasting glucose and insulin available at baseline and a much longer follow-up up to 10 years (average 7.2 years).

To investigate the relationships that might underlie the effect of diabetes on the risk of Alzheimer disease, we investigated whether fasting glucose and insulin levels, and insulin resistance are associated with the risk of Alzheimer disease in persons without diabetes, and if this risk remains the same over a longer follow-up period.

METHODS

The Rotterdam Study

The Rotterdam Study is a prospective population-based cohort study that is conducted among all inhabitants aged 55 years and over of Ommoord, a district of Rotterdam, the Netherlands.⁸ Of 10,274 eligible subjects, 7983 (78%) participated in the baseline examinations between 1990-1993. The medical ethics committee at Erasmus University of Rotterdam approved the study and written informed consent was obtained from all participants. Follow-up examinations were conducted in 1993 to 1994, 1997 to 1999 and 2002 to 2004. In addition, through linkage with records of general practitioners, the total cohort was continuously monitored for morbidity and mortality.

Study population

This study was based on the third survey (1997-1999) of the Rotterdam Study in which fasting blood samples were obtained. Of the 5990 participants of the original cohort who were still alive at that time, 4797 participated, 3795 of whom had fasting blood samples drawn. Of these participants, 3432 were free from dementia and had complete data on glucose and insulin levels, cognitive function and cardiovascular risk factors, including waist circumference, blood pressure, triglycerides and HDL-cholesterol. We excluded 313 participants who had a history of diabetes at the time of the third survey. This resulted in a study population of 3139 subjects.

Glucose and insulin assessments

Fasting blood serum was drawn during the examination at the research center. The blood was stored at -80°C in a number of 5 mL aliquots. Glucose levels were measured within one week of sampling using the glucose hexokinase method.⁹ The remaining serum was kept frozen for later analyses. In 2008, stored serum that had not been thawed previously was used for insulin measurements. Serum insulin was determined by metric assay (Biosource Diagnostics, Camarillo, CA, USA). This assay has no cross-reactivity with either proinsulin or C-peptide. All measurements were done at the clinical chemistry laboratory at Erasmus Medical Center in Rotterdam.

Insulin resistance

Data on fasting glucose and fasting insulin levels were used to calculate the degree of insulin resistance according to the homeostasis model assessment (HOMA).¹⁰ The HOMA index, which is calculated by dividing the product of fasting levels of glucose and insulin by a constant, has been shown to correlate well with the euglycemic hyperinsulinemic clamp method,¹¹ which is the golden standard for measuring insulin resistance.

Dementia case finding

The diagnosis of dementia was made following a three-step protocol.¹² Two brief tests of cognition (Mini-Mental State Examination (MMSE)¹³ and Geriatric Mental State schedule (GMS)¹⁴ organic level) were used to screen all participants. Screen-positives (MMSE score < 26 or GMS organic level > 0) underwent the Cambridge examination for mental disorders of the elderly (Camdex).¹⁵ Participants 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 digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.¹² The diagnoses of dementia and Alzheimer disease were made in accordance with internationally accepted criteria for dementia (DSM-III-R),¹⁶ and Alzheimer disease (NINCDS-ADRDA),¹⁷ by a panel of a neurologist, neuropsychologist and research physician. The follow-up with regard to dementia diagnosis was virtually complete (98.6%) until 1 January 2007.

History of diabetes

History of diabetes was defined as: 1) self-reported history of diabetes (reported use of oral antidiabetes medication, or insulin use, or treatment by diet), 2) registration by a general practitioner as having diabetes, or 3) a previous diagnosis of diabetes during the 1990-1993 or 1993-1994 examinations (based on a random non-fasting plasma glucose ≥ 11.1 mmol/L).¹⁸

Newly diagnosed diabetes

Newly diagnosed diabetes was defined as a fasting glucose level ≥ 7.0 mmol/L in participants without a history of diabetes.

Covariates

Educational level was assessed during the first interview that took place between 1990 and 1993, and was dichotomized into primary education (with or without an unfinished higher education) versus lower vocational to university education. *APOE* genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis. *APOE* $\epsilon 4$ -status was defined as carriership of one or two $\epsilon 4$ -alleles.

Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in a sitting position. The waist circumference was measured in centimeters. HDL-cholesterol levels were measured in fasting serum within one week of the visit to the research center. Serum levels of triglycerides were measured in 2008 at the same time the insulin levels were measured.

Statistical analyses

Because of a non-normal distribution of insulin levels and HOMA we used a \log_2 -transformation, which means that every unit increase on the logarithmic scale reflects a doubling of the absolute values.

We used Cox proportional hazards models to examine the associations of fasting levels of glucose and insulin, and insulin resistance with the risk of developing Alzheimer disease during follow-up.

All analyses were adjusted for age and sex and additional adjustments were made for level of education, *APOE* $\epsilon 4$ -status, and the components of the metabolic syndrome (waist, systolic and diastolic blood pressure, triglycerides and HDL-cholesterol).¹⁹

We added a time-dependent covariate to the model to test the proportionality assumption. Because this assumption was violated we performed the analyses in three different strata according to follow-up time: 1) short follow-up (maximum three years) 2) medium follow-up (3-5.5 years) and 3) long follow-up (5.5-9.7 years). Cut-offs were chosen to have approximately equal numbers of incident cases within the follow-up intervals. We repeated the analyses in strata of *APOE* $\epsilon 4$ -carriership. To test for interaction of *APOE* $\epsilon 4$ -status on the relationship of levels of glucose, insulin, and insulin resistance with the risk of Alzheimer disease we first added an interaction term with *APOE* $\epsilon 4$ -status to the different models. This is the method that is usually performed to test for interaction, and assesses interaction on a multiplicative scale. This, however, measures differences in rate ratios. When biological interaction occurs, one would expect to see interaction on an additive scale, which measures differences in absolute risks.^{20,21} We used the relative risk due to interaction (RERI) to test for interaction on an additive scale.²² Although the number of Alzheimer cases was small in the younger age group, we additionally performed stratified analyses by age median to test whether the associations of levels of glucose and insulin, and insulin resistance with the risk of Alzheimer disease are different for younger versus older people.

All analyses were done using the SPSS statistical package 15.0 (SPSS inc., Chicago, Illinois) or SAS 9.2.

RESULTS

The baseline characteristics of the study population are shown in Table 1. After a follow-up of 22,494 person-years (mean 7.2 years, SD 2.1) 211 participants developed Alzheimer disease, 71 of them within three years of baseline, 72 between three and 5.5 years and 68 after 5.5 years. When we performed the analyses with the total

Table 1. Baseline characteristics of the study population

	N=3139
Age (years)	71.8 ± 6.7
Women (%)	58
Primary education only (%)	29
MMSE (points)	27.8 ± 1.9
Waist (cm) men	97 ± 9
women	90 ± 12
ApoE ε4-allele present (%)	28
Systolic blood pressure (mmHg)	143 ± 21
Diastolic blood pressure (mmHg)	75 ± 11
HDL-cholesterol (mmol/L)	1.41 ± 0.40
Triglycerids (mmol/L)	1.48 ± 0.71
Fasting glucose levels (mmol/L)	5.65 ± 0.91
Fasting insulin levels (mU/L)*	9.3 (6.6-13.3)
Insulin resistance (HOMA)*	2.26 (1.56-3.36)

Data are presented as mean ± standard deviation or percentages

* Median (interquartile range) are presented because of a skewed distribution

follow-up period, fasting levels of glucose and insulin, and insulin resistance were not associated with the risk of Alzheimer disease. (Data not shown)

Table 2 shows the associations of fasting insulin and glucose levels, and insulin resistance, with the risk of Alzheimer disease in the different follow-up strata. Fasting levels of glucose were not associated with the risk of Alzheimer disease after a short or medium follow-up time. There was a relation between higher levels of glucose and a lower risk of Alzheimer disease after 5.5 years (HR per SD increase 0.61; 95% CI 0.41-0.89).

Table 2. Hazard ratios (with corresponding 95% confidence intervals) of the association of fasting glucose and insulin levels, and insulin resistance with the risk of Alzheimer disease according to time-to-event

	Short follow-up (max 3 years) N=3139		Medium follow-up (3-5.5 years) N=2881		Long follow-up (5.5-9.7 years) N=2566	
	HR (95% CI) ¹	HR (95% CI) ²	HR (95% CI) ¹	HR (95% CI) ²	HR (95% CI) ¹	HR (95% CI) ²
Glucose (per SD)	1.08 (0.86-1.35)	1.16 (0.93-1.45)	0.79 (0.58-1.08)	0.85 (0.61-1.18)	0.70 (0.51-0.96)	0.61 (0.41-0.89)
Insulin (per log ₂)	1.12 (0.83-1.51)	1.41 (1.02-1.96)	0.65 (0.48-0.89)	0.71 (0.49-1.02)	0.83 (0.60-1.14)	0.74 (0.51-1.08)
Insulin resistance (HOMA per log ₂)	1.12 (0.85-1.46)	1.39 (1.04-1.86)	0.68 (0.51-0.89)	0.72 (0.52-1.01)	0.80 (0.60-1.07)	0.70 (0.50-0.99)

¹ Adjusted for age and sex

² Adjusted for age, sex, level of education, APOE ε4-status, systolic blood pressure, diastolic blood pressure, HDL-cholesterol, triglycerids and waist circumference

Table 3. Hazard ratios (with corresponding 95% confidence intervals) of the association of fasting glucose and insulin levels, and insulin resistance with the short-term risk of Alzheimer disease according to *APOE* ϵ 4-carriership, with p-values for interaction

	<i>APOE</i> ϵ 4 carriers	<i>APOE</i> ϵ 4 non-carriers	P-values for interaction	
	HR (95% CI)	HR (95% CI)	multiplicative	additive
Glucose (per SD)	0.91 (0.49-1.67)	1.25 (1.03-1.52)	0.165	0.751
Insulin (per log ₂)	1.16 (0.65-2.06)	1.75 (1.16-2.64)	0.087	0.988
Insulin resistance (HOMA per log ₂)	1.10 (0.66-1.84)	1.74 (1.21-2.51)	0.050	0.519

Adjusted for age, sex, level of education, systolic blood pressure, diastolic blood pressure, HDL-cholesterol, triglycerids and waist circumference

Fasting levels of insulin and insulin resistance were associated with a higher short-term risk of Alzheimer disease with an increase in risk of approximately 40% per doubling of insulin levels and insulin resistance in the fully adjusted model. Higher levels of insulin and insulin resistance were no longer associated with an increased risk of Alzheimer disease after three years, and the relation even seemed to invert, reaching borderline significance in some of the fully adjusted models.

Exclusion of 162 participants who had newly diagnosed diabetes based on their fasting glucose levels (≥ 7.0 mmol/L) did not alter the results.

When we applied the fully adjusted model in strata of *APOE* ϵ 4-carriership, levels of glucose and insulin, and insulin resistance were all associated with the short-term risk of Alzheimer disease in non-carriers of the *APOE* ϵ 4-allele. (Table 3) In these non-carriers, doubling of levels of insulin and insulin resistance increased the short-term risk of AD increased by 75%. In carriers of the *APOE* ϵ 4-allele, higher levels of glucose, insulin and insulin resistance had no apparent effect on the short-term risk of Alzheimer disease. There was no significant interaction of *APOE* ϵ 4-status and glucose levels, insulin levels and insulin resistance on an additive scale, and a borderline significant interaction of *APOE* ϵ 4-status and insulin and insulin resistance on a multiplicative scale.

The risk of Alzheimer disease after a medium or long follow-up did not differ between carriers and non-carriers of the *APOE* ϵ 4-allele (p-values for multiplicative and additive interaction >0.25).

In the stratified analyses by age median (71.0 years) there was no effect of glucose levels on the risk of Alzheimer disease, and higher insulin levels and insulin resistance were also not associated with an increased risk of Alzheimer disease after three years of follow-up in both age groups.

DISCUSSION

In this large population-based cohort study we found that higher levels of fasting insulin and insulin resistance are associated with a higher short-term risk of Alzheimer disease, but that after three years the risk is no longer increased; if anything, the relation even seems to invert. Levels of glucose are not associated with a higher risk of Alzheimer disease and even seem beneficial after several years of follow-up. There is no evidence for a biological interaction between *APOE* ϵ 4-status and levels of glucose, insulin and insulin resistance on the risk of Alzheimer disease.

The major strengths of our study are the prospective population-based design, the large number of participants and the long follow-up period with almost no loss to follow-up. Especially the latter is important as theoretically the strength of a longitudinal association may diminish or even invert if there is selective attrition of people who develop dementia over the follow-up period. We are confident however that through our very dense and complete case-finding and follow-up, selective attrition cannot explain the findings in our study.

Unfortunately, we had no repeated glucose and insulin measurements over time. Therefore we could not assess whether changes in glucose and insulin levels have an impact on the longer term risk of Alzheimer disease and we might underestimate the longer term risk of Alzheimer disease.

We considered whether the assessment of insulin levels after 10 years of storage at -80°C might have affected the stability of the samples. Although we are not aware of a study on the stability of insulin levels after long-term storage of serum, there are several studies on the stability of other proteins and hormones which show that long-term storage at -25°C and especially -80°C does not affect the stability of the measurements, and that the expected stability of materials stored at -80°C is at least 10 years.²³⁻²⁵ Furthermore, any change in insulin levels after 10 years of storage should affect all samples, without any relation with our outcome Alzheimer disease. Because we had no fasting blood samples at the start of the Rotterdam Study, we used the third survey as the baseline for the current study. This was a selection of more healthy participants, with less cognitive deficits and less diabetes, who came to the research center and had fasting blood drawn. However, since we excluded prevalent Alzheimer patients and participants with a history of diabetes, we consider it unlikely that this selection will have influenced our results.

Our results show that characteristics related to diabetes, especially higher levels of insulin and insulin resistance, are associated with a higher risk of Alzheimer disease only within three years. This supports our previous finding of an association of diabetes with a higher risk of Alzheimer disease with an average follow-up of 2.1 years.² Another recent study from our cohort showed no association of higher

levels of glucose and insulin resistance with cognitive decline after on average 4.6 years in participants without a history of diabetes. In that study, there was selective non-participation with regard to the outcome of cognitive decline of those who were diagnosed with dementia in between cognitive examinations and did not visit the research center for the follow-up assessment.²⁶ These are the participants who developed dementia mainly within three years of baseline, which is the time period in which we find an increased risk of Alzheimer disease with higher levels of insulin and insulin resistance.

Several other studies have shown associations of the glucose and insulin metabolism with the risk of Alzheimer disease.²⁷⁻³² Most of these studies had an average follow-up time of around 5 years. The studies that have a longer follow-up time often do not find a higher risk of Alzheimer disease with disturbances in the insulin metabolism. A recent study from the Uppsala Longitudinal Study of Adult Men showed no association of six out of seven measures of the insulin metabolism including fasting insulin levels and insulin sensitivity, determined by the euglycemic insulin clamp method, with the risk of Alzheimer disease after a median follow-up of 12 years. A low early insulin response to oral glucose challenge was however associated with a higher risk of Alzheimer disease.²⁹ In the Framingham study after a long follow-up period of on average 12.7 years, diabetes did not increase the overall risk of dementia and Alzheimer disease.³³ If we look at the risk of Alzheimer disease over the total follow-up period in our study, without dividing the time-to-event into strata, we also find no association of our three measures and the risk of Alzheimer disease.

The higher short term risk of Alzheimer disease which disappears after three years of follow-up might be explained by several mechanisms. First, since the underlying neuropathological changes that cause Alzheimer disease are already ongoing years before the clinical diagnosis can be made, our results might reflect reversed causality or a common pathophysiology instead of a causal role of insulin metabolism in Alzheimer disease. Second, we hypothesized that diabetes and disturbances in the insulin metabolism only increase the risk of Alzheimer disease for a short time period by advancing the onset in those already on the verge of getting Alzheimer disease. This would mean that disturbances in the insulin metabolism are not a cause of the neuropathological changes that cause Alzheimer disease, but do influence and accelerate those changes, leading to an earlier onset of Alzheimer disease.

We found that after three years of follow-up the risk of Alzheimer disease was no longer increased and even inverted. It seems very unlikely that the inversion of the effect on Alzheimer disease risk reflects a true biological mechanism. More likely, competing risks might explain these findings: people with diabetes or pre-diabetes are at higher risk of cardiovascular diseases and death, which makes them no longer at risk of Alzheimer disease, especially in the long-term.

Although there was a difference in the effect size on the short-term risk between carriers and non-carriers of the *APOE* ϵ 4-allele, there was no interaction on an additive scale. The borderline significant negative interaction on a multiplicative scale suggests that the differences in risk ratio can be explained by the differences in baseline absolute risks, and not by a true interaction between *APOE* ϵ 4-status and insulin metabolism.²⁰

Different studies report conflicting results on the influence of the *APOE* ϵ 4-allele on the relation between diabetes or insulin metabolism and the risk of Alzheimer disease. A number of studies report higher effect estimates in non-carriers of the *APOE* ϵ 4-allele, although none of these studies report if they have tested for additive interaction.^{32,34,35} The Uppsala Longitudinal Study of Adult Men showed a stronger effect of a low acute or early insulin response, assessed at midlife²⁸ and at age 71²⁹ on the risk of Alzheimer disease in non-carriers of the *APOE* ϵ 4-allele. However, in the same study, higher levels of fasting insulin and HOMA, both assessed at midlife, were associated with the risk of Alzheimer disease only in carriers of the *APOE* ϵ 4-allele, which is conflicting with our results.²⁸ We assessed our measurements at an older age, which might explain these differences. Several other studies also showed a stronger effect of diabetes and hyperinsulinemia on the risk of Alzheimer disease in carriers of the *APOE* ϵ 4-allele.^{31,36,37} Investigators from the Kungsholmen Project initially reported an effect of diabetes on the risk of Alzheimer disease only in carriers of the *APOE* ϵ 4-allele³⁷, yet in a later study they found an effect of borderline diabetes, defined as a random plasma glucose level of 7.8-11.0 mmol/L, on the risk of Alzheimer disease only in non-carriers.³⁸ These discrepant results fit our finding of no interaction between *APOE* ϵ 4-status and insulin metabolism on the risk of Alzheimer disease.

In conclusion, higher levels of insulin and insulin resistance were associated with a higher short-term risk of Alzheimer disease. Glucose and insulin levels and insulin resistance were not associated with the risk of Alzheimer disease after three years. There was no interaction of the insulin metabolism and *APOE* ϵ 4-status on the risk of Alzheimer disease. Our findings suggest that the insulin metabolism influences the clinical manifestation of Alzheimer disease only within a short time-period, possibly by advancing the onset in those already on the verge of getting AD.

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Chapter 5.2

Associations of serum cortisol with cognitive function and dementia

Elisabeth M.C. Schrijvers, Nese Direk, Peter J. Koudstaal, Clemens Kirschbaum,
Albert Hofman, Henning Tiemeier, Monique M.B. Breteler

ABSTRACT

Higher levels of cortisol have been observed in persons with cognitive decline and dementia. It is unknown whether these higher levels are a cause or a consequence of disease. We investigated whether morning levels of serum cortisol were associated with cognitive function, cognitive decline, and the risk of dementia and Alzheimer disease in the Rotterdam Study, a large prospective population based cohort study. Cortisol levels were assessed in fasting blood serum in 3341 participants, who were free of dementia at baseline (1997-1999). Cognitive function was assessed with a dedicated neuropsychological test battery at baseline and at follow-up examination (2002-2004). In addition, the cohort was continuously monitored for incident dementia until January 1, 2007. After a mean follow-up of 7.1 years, 243 participants had developed dementia, of whom 210 were diagnosed with Alzheimer disease. Morning serum levels of cortisol were neither related to cognitive function at baseline, nor to annual cognitive decline. There was no relation between serum levels of cortisol and the risk of developing dementia or Alzheimer disease. These results suggest that that morning serum cortisol is not a causal factor in the development of dementia.

INTRODUCTION

Cortisol is a stress hormone within the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis follows a diurnal pattern with the highest cortisol production in the second half of the night, a peak increase within 30 minutes after awakening and a steady decline throughout the rest of the day.^{1,2} In response to stressful conditions cortisol mediates many metabolic processes such as energy utilization, enhancement of cardiovascular output, increase in cerebral perfusion and modulation of the immune system.^{1,3} A prolonged stress response with chronic elevation of cortisol has been associated with various chronic diseases and metabolic changes in the body.^{3,4} Disturbances on cortisol release have been described in dementia and cognitive impairment.⁵⁻⁸ It is unknown whether these disturbances are a cause or consequence of disease.⁹ On the one hand, higher levels of cortisol are associated with hippocampal atrophy, which can affect memory performance and lead to dementia.^{10,11} On the other hand, damage to the hippocampus may lead to reduced inhibition of the HPA axis and thus to higher serum cortisol levels.¹²

Most studies that have investigated the relation between cortisol and cognition or dementia were cross-sectional and therefore inconclusive regarding the temporal relation between levels of cortisol and dementia.^{5,6,8,10,13-16} The studies that did have a longitudinal design did not investigate the risk of developing dementia.^{5,7,17-20} Furthermore, results have been very inconsistent.

We investigated in a large population-based cohort study of non-demented elderly whether morning levels of serum cortisol are associated with cognitive function, cognitive decline, and the risk of developing dementia.

METHODS

Study population

The Rotterdam Study is a prospective population-based cohort study that is conducted among all inhabitants aged 55 years and over of Ommoord, a district of Rotterdam, the Netherlands.²¹ The medical ethics committee at Erasmus University of Rotterdam approved the study and after complete description of the study to the participants, written informed consent was obtained from all participants.

Of 10,274 eligible subjects, 7983 (78%) participated in the baseline examinations between 1990 and 1993. Follow-up examinations were conducted in 1993 to 1994, 1997 to 1999 and 2002 to 2004. In addition, through linkage with records of general practitioners, the total cohort was continuously monitored for morbidity and mortality.

This study was based on the third survey (1997-1999) of the Rotterdam Study. Of the 5990 participants of the original cohort that were still alive at that time, 4797 participated, 3795 of whom had fasting blood samples drawn. Cortisol measurements were available in 3418 participants who were free from dementia. We excluded 74 participants who were using systemic corticosteroids, and three participants who had their blood drawn after 11 AM. This resulted in a study population of 3341 subjects who were followed for incident dementia. From these participants, 3006 were still alive at the start of the fourth survey and 2524 visited the research center. Data on at least one test from the neuropsychological test battery were available in 2512 participants.

Serum cortisol assessment

Fasting blood serum was obtained at the research center between 8 and 11 AM. The blood was stored at -20°C in a number of 5 mL aliquots. The serum was kept frozen for later analyses. In 2010, stored serum that had not been thawed previously was sent to the Technical University of Dresden, Germany for analysis. Serum cortisol was measured by luminescence immunoassay (IBL, Hamburg, Germany). Intra-assay and interassay coefficients of variation were below 6%.

Dementia case finding

Participants were screened for dementia at baseline and follow-up visits using a three-step protocol.²² Two brief tests of cognition (Mini-Mental State Examination (MMSE)²³ and Geriatric Mental State schedule (GMS)²⁴ organic level) were used to screen all participants. Screen-positives (MMSE score < 26 or GMS organic level > 0) underwent the Cambridge examination for mental disorders of the elderly (Camdex).²⁵ Participants 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 digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.²² The diagnoses of dementia and Alzheimer disease were made in accordance with internationally accepted criteria for dementia (DSM-III-R)²⁶, and Alzheimer disease (NINCDS-ADRDA)²⁷, by a panel of a neurologist, neuropsychologist and research physician. The follow-up with regard to dementia diagnosis was virtually complete (98.7%) until January 1, 2007.

Cognitive function

Global cognitive function was assessed with the MMSE. In addition, a dedicated neuropsychological test battery was used to assess executive function, attention and information processing speed. The test battery included the Letter-Digit Substitution Task (LDST)²⁸, the Word Fluency Test (WFT)²⁹ and the abbreviated Stroop test.³⁰ A specific test to assess memory was not implemented until the fourth survey (2002-2004).

Covariates

Educational level was assessed during the first home-interview that took place between 1990 and 1993, and was dichotomized into primary education (with or without an unfinished higher education) versus lower vocational to university education. APOE genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis. APOE ϵ 4-status was defined as carriership of one or two ϵ 4-alleles. At the third survey, blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in a sitting position. The waist circumference was measured in centimeters. Serum glucose and HDL-cholesterol levels were measured in fasting serum within one week of the visit to the research center. Smoking status was assessed during the home-interview, which took place before the visit to the research center. Depressive symptoms were assessed during the home-interview by use of a depression questionnaire: the 20-item version of the Center for Epidemiological Studies Depression scale (CES-D) with a cut-off score of 16.³¹ Missing values in covariates (<2%) were imputed based on age and gender for normal distributed variables and as the mean for non-normal distributed variables and dichotomous variables. Missing values in APOE ϵ 4-status (N=134, 4%) were not imputed.

Statistical analyses

First, we investigated the cross-sectional association of serum cortisol with cognitive function, using linear regression models. After establishing that cortisol followed a normal distribution, cortisol was entered continuously per standard deviation (SD) increase into the models. We also entered cortisol per quartile of its distribution to check for a non-linear relation of cortisol with cognitive function and decline, and dementia. All analyses were adjusted for age and sex and additional adjustments were made for time of blood sampling, level of education, presence of depressive

symptoms, waist circumference, systolic and diastolic blood pressure, serum glucose, HDL-cholesterol, and current smoking status.

Second, we used linear regression analyses to investigate the longitudinal associations of serum cortisol with cognitive decline, with the annual change of cognitive function as dependent and serum cortisol as independent variables. Annual cognitive decline was calculated as the difference between the test scores at the 4th and the 3rd survey divided by follow-up time. The analyses were adjusted for the abovementioned covariates and we added a third model with an additional adjustment for baseline test score.

Third, we used Cox proportional hazards models to examine the association of levels of cortisol with the risk of developing dementia and Alzheimer disease during follow-up. The analyses were adjusted for the abovementioned covariates. We added a time dependent covariate to the model to test if the hazards were constant over time. Because this covariate was significant ($p=0.004$), indicating that the association between cortisol and dementia differed according to length of follow-up, we performed the analyses in two different strata according to follow-up time: 1) short follow-up (0-4.8 years) 2) long follow-up (4.8-9.6 years).

We repeated the analyses in strata of *APOE* $\epsilon 4$ -carriership and we tested for interaction of *APOE* $\epsilon 4$ -carriership by adding *APOE* $\epsilon 4$ -status and the product of *APOE* $\epsilon 4$ -status with cortisol to the unstratified models.

All analyses were done using the SPSS statistical package 15.0 (SPSS inc., Chicago, Illinois).

RESULTS

Baseline characteristics of the study population are shown in Table 1. During a mean follow-up of 7.1 years (range 0.1-9.6) 243 participants developed dementia, of whom 210 were diagnosed with Alzheimer disease. The remaining 33 cases were ascribed to other subtypes of dementia (including vascular dementia, dementia in Parkinson's disease, multi system atrophy and Lewy body dementia). The mean time between the two surveys with the assessments of the neuropsychological test battery was 4.6 years (SD 0.5).

Serum cortisol was not associated with the performance on the different cognitive tests in the cross-sectional analyses (Table 2), nor with the annual change in test scores (Table 3). There was no evidence for a non-linear relation of serum cortisol with cognitive test score and annual decline.

Cortisol was also not associated with the risk of incident dementia in the overall follow-up (hazard ratio (HR) per SD increase 1.01, 95% confidence interval (CI) 0.89-

Table 1. Baseline characteristics of the study population

	N=3341
Age (years)	72.0 ± 6.8
Female	1921 (58)
Only primary education	955 (29)
Current smokers	527 (16)
Depressive symptoms (≥16 pts CES-D)	203 (6)
MMSE (points)	27.7 ± 1.9
Carrier of an <i>APOE</i> ε4-allele	901 (27)
Systolic blood pressure (mmHg)	143 ± 21
Diastolic blood pressure (mmHg)	75 ± 11
Waist (cm) men	97.4 ± 9.4
women	90.2 ± 12.0
Serum glucose (mmol/L)	5.89 ± 1.32
HDL cholesterol (mmol/L)	1.40 ± 0.40
Serum cortisol (nmol/L)	304 ± 94

Data are presented as mean ± standard deviation or numbers (percentages)

Table 2. Differences in cognitive test performance at baseline per standard deviation increase in serum cortisol levels

	MMSE (points) N=3337		LDST (points) N=3264		Word fluency (points) N=3302		Stroop trial 3 (seconds) N=3222	
	Difference	95% CI	Difference	95% CI	Difference	95% CI	Difference	95% CI
Model 1	-0.021	-0.084; 0.042	-0.049	-0.265; 0.167	0.047	-0.130; 0.223	0.066	-0.612; 0.744
Model 2	-0.042	-0.108; 0.024	-0.079	-0.301; 0.143	0.075	-0.111; 0.262	0.086	-0.626; 0.798

MMSE=mini mental state examination, LDST= letter digit substitution task,

Model 1: adjusted for age and sex

Model 2: 1+ adjusted for education, time of blood sampling, depressive symptoms, waist circumference, systolic and diastolic blood pressure, serum glucose, HDL cholesterol and current smoking status

1.15), neither in the short follow-up period (HR 1.17, 95% CI 0.98-1.40), nor in the long follow-up period (HR 0.87, 95% CI 0.72-1.05). Further adjustments for education, depressive symptoms and cardiovascular risk factors did not change the results. The associations of serum cortisol level and risk of Alzheimer disease were similar to those for overall dementia (Table 4). There was no evidence for a non-linear relation of serum cortisol with the risk of incident dementia or Alzheimer disease.

The associations were not different for participants with or without at least one *APOE* ε4-allele (p-values for interaction>0.09).

Table 3. Differences in annual change in test score per standard deviation increase in serum cortisol levels

	MMSE (points) N=2512		LDST (points) N=2344		Word fluency (points) N=2420		Stroop trial 3 (seconds) N=2202	
	Difference	95% CI	Difference	95% CI	Difference	95% CI	Difference	95% CI
Model 1	-0.012	-0.031; 0.007	0.026	-0.010; 0.062	0.018	-0.024; 0.061	0.131	-0.036; 0.298
Model 2	-0.010	-0.031; 0.011	0.018	-0.021; 0.057	0.002	-0.044; 0.048	0.105	-0.075; 0.285
Model 3	-0.007	-0.027; 0.013	0.016	-0.022; 0.054	0.011	-0.030; 0.052	0.128	-0.047; 0.303

MMSE = Mini Mental State Examination, LDST = Letter Digit Substitution Task

Model 1: adjusted for age and sex

Model 2: 1+ adjusted for education, time of blood sampling, depressive symptoms, waist circumference, systolic and diastolic blood pressure,

serum glucose, HDL cholesterol and current smoking status

Model 3: 2+ adjusted for baseline test score

Table 4. Risk of dementia and Alzheimer disease per standard deviation increase in serum cortisol levels according to time-to event (N=3341)

	Total follow-up				Within 4.8 years				After 4.8 years			
	Dementia n=243		AD n=210		Dementia n=122		AD n=107		Dementia n=121		AD n=103	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Model 1	1.01	0.89-1.15	1.00	0.87-1.15	1.17	0.98-1.40	1.14	0.94-1.38	0.87	0.72-1.05	0.86	0.70-1.06
Model 2	1.00	0.87-1.15	0.99	0.85-1.14	1.17	0.96-1.41	1.15	0.94-1.42	0.85	0.69-1.03	0.83	0.67-1.03

AD = Alzheimer disease, N = total number of participants in the analyses, n= number of cases

Model 1: adjusted for age and sex

Model 2: 1+ adjusted for education, time of blood sampling, depressive symptoms, waist circumference, systolic and diastolic blood pressure, serum glucose, HDL cholesterol and current smoking status

DISCUSSION

In this large population based cohort study morning serum levels of cortisol were neither associated with cognitive function or cognitive decline, nor with the risk of dementia.

We looked at two different follow-up periods for incident dementia, because the time dependent covariate indicated that the association between cortisol and dementia differed according to follow-up time. We found, however, no association in either follow-up period, and the difference between the two periods can be explained by the fact that the hazard ratio in the first period was slightly and non-significantly larger than 1, while the hazard ratio in the second period was slightly and non-significantly smaller than 1. Due to our large numbers and long follow-up time, this difference became significant after statistical testing.

Previously, several studies have reported that blood or cerebrospinal fluid (CSF) levels of cortisol were higher in Alzheimer patients compared with controls.^{5,8,13,14}

These studies were all cross-sectional and therefore not able to establish a temporal relationship between cortisol and dementia. One study found no baseline differences in serum cortisol levels between eight Alzheimer patients and four controls, but the Alzheimer patients had higher serum cortisol levels during the hours after undergoing a lumbar puncture, possibly due to an exaggerated stress response or a relative reduction in HPA negative feedback.¹⁵ The studies that also reported cortisol levels in patients with mild cognitive impairment (MCI), found no increase of cortisol levels in MCI patients compared to controls, indicating that increased cortisol levels do not occur early in the development of Alzheimer disease and might be the result, rather than the cause of the disease.^{8,14} In line with these observations, our results from a prospective cohort suggest that morning serum levels of cortisol are not causally related to the development of dementia and Alzheimer disease.

The results of studies investigating the association between cortisol and cognitive function or cognitive decline have been inconsistent. This might be partly due to differences in assessment of cortisol, e.g. saliva, blood or CSF sampling, and measurements at different times during the day. Several studies reported an association of higher cortisol levels with worse cognitive function.^{6,10,19,20} In contrast, a recent study found that higher morning levels of cortisol were associated with better memory performance in healthy elderly, with poorer memory performance in MCI patients and not with the memory performance in Alzheimer patients.¹⁶ A study reporting data from a population-based cohort found that high morning serum levels of free cortisol were associated with poorer performance on verbal learning, and in a women also with slower speed of information processing, which is conflicting with our results.¹⁷ The same study, however, reported no association of higher levels of cortisol with cognitive decline over a period of 6 years, which is in line with our results. In another study from the same cohort, where the investigators assessed both morning and evening levels of cortisol in saliva, overall, there was also no association between levels of cortisol and cognitive decline over 4 years of follow-up. However, in a subgroup analysis according to *APOE* ϵ 4-carriership, there was an association between lower morning cortisol levels, higher evening cortisol levels, and flattened diurnal variability with an increased risk for memory decline in *APOE* ϵ 4-carriers, but not in non-carriers.¹⁸ Other longitudinal studies reported associations of higher levels of cortisol and more cognitive decline in Alzheimer patients^{5,19}, a slower decline in MCI patients⁷ and no association in normal controls.^{5,7} We did not have enough Alzheimer patients who completed the cognitive test battery at both time points to investigate whether higher levels of cortisol were related to a more rapid decline in Alzheimer patients. A population based study in people of 85 years at baseline did show an association between higher morning levels of plasma cortisol and cognitive decline during a mean follow-up of 4.2 years.²⁰ In that study, however, participants

were older than our population and participants with dementia at baseline were not excluded, making it difficult to compare the results with our study. Overall, studies have not convincingly shown an association of higher levels of cortisol with worse cognitive function or more cognitive decline over time. This supports our finding of no association between serum cortisol with cognitive function, cognitive decline and incident dementia.

Important strengths of our study are the large number of participants and the long follow-up, which was virtually complete with regard to the dementia diagnosis. Especially the latter is important as in studies that only investigate cognitive decline measured by repeated cognitive testing there will be selective drop-out at follow-up examinations of participants with more cognitive decline. Because we investigated not only cognitive decline, where drop-out might influence the results, but also the risk of developing dementia, our conclusions are more robust. Furthermore, given the large number of participants and dementia cases, and the small confidence intervals, we believe our study has enough power to conclude that morning serum levels of cortisol were not associated with cognitive function and decline or the risk of developing dementia. The study was based within the general population, which increases the external validity and decreases potential selection bias. Additionally, we were able to take into account many possible covariates which may affect the estimates.

Unfortunately, we could not include memory function or decline in memory function in our analyses because a specific test to assess memory was not implemented until the fourth survey. Memory is one of the main cognitive domains affected by hippocampal damage and memory decline is often one of the first signs in the development of Alzheimer disease.

Like many other studies, we measured cortisol at one time point in the morning only.^{5,8,13,14,16,17,19,20} A single measurement does not reflect the circadian rhythm of cortisol secretion, and it would be preferable to have at least two measurements on one day. However, fasting serum cortisol has been shown to have low intraindividual variability and to correlate well with the feedback sensitivity of the HPA axis.³² Another limitation in our study is that we did not have information on awakening time. However, morning serum cortisol levels in our study are well within normal limits (138-690 nmol/L) for this time of the day.³³ We also considered whether the long-term storage of our serum could have affected the cortisol levels. We considered this unlikely, because cortisol is known for its stability after years, and even after decades of storage.^{34,35}

Finally, we can only draw conclusions on the lack of an association between morning levels of serum cortisol with cognition and dementia. How, and to what extent the HPA axis, which is more complex than can be measured by peripheral cortisol

levels, is involved in the pathogenesis of cognitive decline and dementia remains undetermined.

In conclusion, morning serum levels of cortisol were not associated with cognitive function and decline or the risk of dementia in an elderly population, suggesting that morning serum cortisol is not a causal factor in the development of dementia.

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6

Genome-wide association study of vascular
dementia

Chapter 6

Genome-wide association study of vascular dementia

Elisabeth M.C. Schrijvers, Britta Schürmann, Peter J. Koudstaal,
Hendrik van den Bussche, Cornelia M. Van Duijn, Frank Hentschel, Reinhard Heun,
Albert Hofman, Frank Jessen, Heike Kölsch, Johannes Kornhuber, Oliver Peters,
Fernando Rivadeneira, Eckart Rüther, André G. Uitterlinden, Steffi Riedel-Heller,
Martin Dichgans, Jens Wiltfang, Wolfgang Maier, Monique M.B. Breteler,
M. Arfan Ikram

ABSTRACT

Background Most studies investigating the genetics of dementia have focused on Alzheimer disease, but little is known about the genetics of vascular dementia. The aim of our study was to identify new loci associated with vascular dementia.

Methods We performed a genome-wide association study in the Rotterdam Study, a large prospective population-based cohort study in the Netherlands. We sought to replicate genome-wide significant loci in two independent replication samples.

Results In the discovery analysis of 5700 dementia-free individuals, 67 persons developed incident vascular dementia over a mean follow-up time of 9.3 ± 3.2 years. We showed genome-wide significance for rs12007229, which is located on the X-chromosome near the androgen receptor gene (OR: 3.7; 95% CI 2.3-5.8, per copy of the minor allele; $p=1.3 \times 10^{-8}$). This association was further confirmed in two independent populations (p-value of combined replication samples=0.024).

Conclusions Our study shows a novel genetic locus for vascular dementia on the X-chromosome.

INTRODUCTION

Vascular dementia is the second most common type of dementia, accounting for approximately 15 to 20% of all cases of dementia.¹ Vascular dementia is defined as the loss of cognitive function resulting from ischemic, ischemic-hypoxic, or hemorrhagic brain lesions as a result of cerebrovascular disease and cardiovascular pathologic changes.² Most studies that investigated the genetics of dementia have focused on Alzheimer disease, the most common type of dementia. Candidate gene studies on vascular dementia often investigated genes implicated in Alzheimer disease³ or focused on rare subtypes of vascular dementia like CADASIL.⁴ Recently, several genome-wide association studies (GWAS) have been done on Alzheimer disease, leading to the discovery of new loci.^{5,6} To identify new loci associated with vascular dementia we performed a GWAS in the Rotterdam Study, a large prospective population-based cohort study. We sought to replicate genome-wide significant loci in two independent populations.

METHODS

Gene discovery

Incident vascular dementia in the Rotterdam Study

The Rotterdam Study is conducted among inhabitants aged 55 years and over of Ommoord, a district of Rotterdam, the Netherlands (N=7983, virtually all Caucasian). Details of the study have been described elsewhere.⁷ The medical ethics committee at Erasmus University Rotterdam approved the study and written informed consent was obtained from all participants. Baseline examinations were conducted in 1990-1993, with follow-up examinations in 1993-1994, 1997-1999 and 2002-2004. Participants were screened for dementia at baseline and follow-up visits using a three-step protocol.⁸ In addition, the cohort was continuously monitored for incident dementia through medical records from general practitioners.⁸ The diagnoses of dementia and vascular dementia were made in accordance with internationally accepted criteria for dementia (DSM-III-R), and vascular dementia (NINDS-AIREN).⁹ Persons with mixed-type dementia (e.g. Alzheimer disease with cerebrovascular disease) were not included in the vascular dementia group. Follow-up for dementia was virtually complete until January 1, 2005.

Genotyping

All persons attending the baseline examination in 1990-1993 consented to genotyping and had DNA extracted. Genotyping was attempted in persons with high-quality extracted DNA (n=6449) and was done using the Illumina Infinium II HumanHap-550chipv3-0® array in 2007-2008 according to the manufacturer's protocols. Samples with low call rate (<97.5%, n=209), with excess autosomal heterozygosity (> 0.336, n=21), with sex-mismatch (n=36), or if there were outliers identified by the IBS clustering analysis (>3 standard deviations from population mean, n=102 or IBS probabilities > 97%, n=129) were excluded from the study population with some persons meeting more than one exclusion criterion.⁶ In total, 5974 samples were available with good quality genotyping data, 42 persons were excluded because they did not undergo cognitive screening, and 232 persons were excluded because they suffered from dementia at baseline. This yielded a discovery population of 5700 persons, who were followed for incident vascular dementia. Because imputation was unavailable for the X-chromosome, unimputed data were used.

Statistical analysis

We used PLINK genetical software to perform 1 degree-of-freedom allelic tests to obtain odds ratios with corresponding 95 % confidence intervals per copy increase of the coded allele. For analysis of the X-chromosome, the allelic test in PLINK considers male carriers equivalent to heterozygote females, i.e. the obtained beta reflects the effect size per allele increase. We used a threshold of 5×10^{-8} for genome-wide significance. We used marker inclusion thresholds of minor allele frequency (MAF)>0.01, SNP call rate>0.90, and Hardy-Weinberg $P > 0.001$.

Replication

Prevalent vascular dementia in the Rotterdam Study

At baseline, 35 persons had vascular dementia. Genotyping of the significant hit from the discovery phase, rs12007229, was unsuccessful in 74 persons without dementia, leaving 35 prevalent cases and 5626 persons without dementia at baseline for the initial replication.

German case-control sample

Patients with vascular dementia and controls were recruited at the Memory Clinics, Department of Psychiatry, University of Bonn and Central Institute of Mental Health, Mannheim (together 160 cases and 160 controls). Additional subjects (27 cases and 29 controls) were recruited at six German gerontopsychiatric university departments in the context of the German Dementia Competence Network DCN-(<http://www.kompetenznetz-demenzen.de>), and another 46 cases and corresponding controls were recruited through primary care in the scope of the German Competence Network of Degenerative Dementias (<http://www.knd-demenzen.de>) from six German recruitment sites. Vascular dementia diagnosis was guided by the NINDS-AIREN criteria and supported by general physical, neurological, and psychiatric examinations, standard laboratory testing and neuroimaging (imaging not performed in the latter 46 cases and controls). Persons with mixed-type dementia were excluded from the study. All patients or their legal guardians gave informed consent for participation in the study. The ethics committees of all the participating universities approved the study. DNA was shipped to Rotterdam for genotyping. The significant hit from the discovery phase, rs12007229, was genotyped using Applied Biosystems Taqman® allelic discrimination assay. For comparability across the discovery and replication study, we excluded five cases and six controls aged <55 years, and seven cases and sixteen controls of whom data on genotyping were missing. This resulted in a study population of 221 cases and 213 controls for the replication in an independent sample.

Statistical analyses

For the replication analyses, we used 1 degree-of-freedom allelic tests with one-sided $p < 0.05$ as threshold for significance. In the Rotterdam Study, the incident discovery analysis is independent from the prevalent replication analysis, because both contribute sets of person-years independent from each other. The independence of these analyses was confirmed in simulation studies.¹⁰

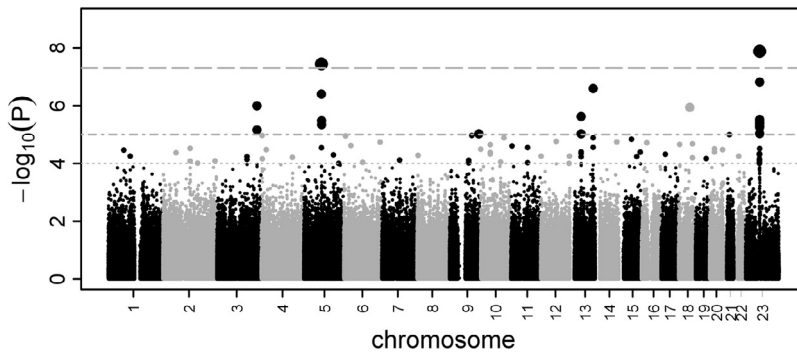
RESULTS

Table 1 shows the age and sex distribution of the different samples. In the discovery analysis of 5700 dementia-free individuals, 67 persons developed incident vascular dementia over an average follow-up time of 9.3 ± 3.2 years. We identified two SNPs that reached genome-wide significance (Figure 1): rs12007229 on the X-chromosome ($p = 1.3 \times 10^{-8}$) and rs10491487 on chromosome 5 ($p = 3.7 \times 10^{-8}$).

Table 1. Baseline characteristics of the discovery and replication samples

	Discovery		Replication I		Replication II	
	Cases	Controls	Cases	Controls	Cases	Controls
N	67	5633	35	5626	221	213
Age, years	77.0 ± 7.4	68.8 ± 8.7	83.6 ± 7.3	68.9 ± 8.8	75.4 ± 7.3	75.4 ± 7.0
Females	33 (49%)	3314 (59%)	26 (74%)	3278 (57%)	125 (57%)	123 (58%)

Data are presented as mean ± standard deviation or numbers (percentages)

**Figure 1.** Genome-wide association plot (Manhattan plot)

Manhattan plot showing all SNPs with their respective chromosome and position on the x-axis plotted against their association p-value on the y-axis. The horizontal lines are for threshold for p-values $<10^{-4}$, $<10^{-5}$, and $<5 \times 10^{-8}$.

We first performed preliminary replication analyses in the prevalent cases of the Rotterdam Study, which in itself was underpowered as a full replication study. Because we found no association between rs10491487 and vascular dementia (OR 1.1, one-sided p-value 0.42), and we did observe an association between rs12007229 and vascular dementia (OR 1.7, one-sided p-value 0.09), we decided to genotype only rs12007229 in the independent German sample.

Table 2 shows the odds ratios for this SNP in the discovery phase and the replication studies separately and combined. We found a significant association of rs12007229 with vascular dementia when we combined both replication samples (OR 1.5, one-sided p-value 0.02). Figure 2 plots p-values from the discovery analysis of SNPs in the vicinity of rs12007229 and shows this locus to be close to the androgen-receptor gene (*AR*).

DISCUSSION

In this GWAS of vascular dementia we identified a new locus related to vascular dementia on the X-chromosome. We replicated this locus in independent populations. The locus on the X-chromosome (rs12007229) is located close to the *AR* gene, which is composed of 8 exons.¹¹ Exon 1 of the *AR*-gene encodes the amino terminal domain,

Table 2. Association of rs12007229 and vascular dementia in the different populations

		Total	Women	Men
Discovery	MAF controls	7.1%	7.0%	7.5%
	MAF cases	22.0%	22.7%	20.6%
	OR (95% CI)	3.7 (2.3-5.8)	3.9 (2.3-6.7)	3.2 (1.4-7.2)
	p-value	1.3×10^{-8}	4.3×10^{-3}	8.4×10^{-7}
Replication I	MAF controls	7.3%	7.2%	7.7%
	MAF cases	11.5%	9.6%	22.2%
	OR (95% CI)	1.7 (0.8-3.8)	1.4 (0.6-3.4)	3.4 (0.7-16.7)
	p-value	0.09	0.25	0.06
Replication II	MAF controls	8.0%	9.3%	6.7%
	MAF cases	11.6%	14.7%	7.3%
	OR (95% CI)	1.5 (0.9-2.4)	1.6 (0.9-2.8)	1.1 (0.4-3.4)
	p-value	0.07	0.06	0.43
Meta-replication	OR (95% CI)	1.5 (1.0-2.4)	1.5 (0.9-2.4)	1.6 (0.7-4.1)
	p-value	0.02	0.05	0.15

MAF= minor allele frequency

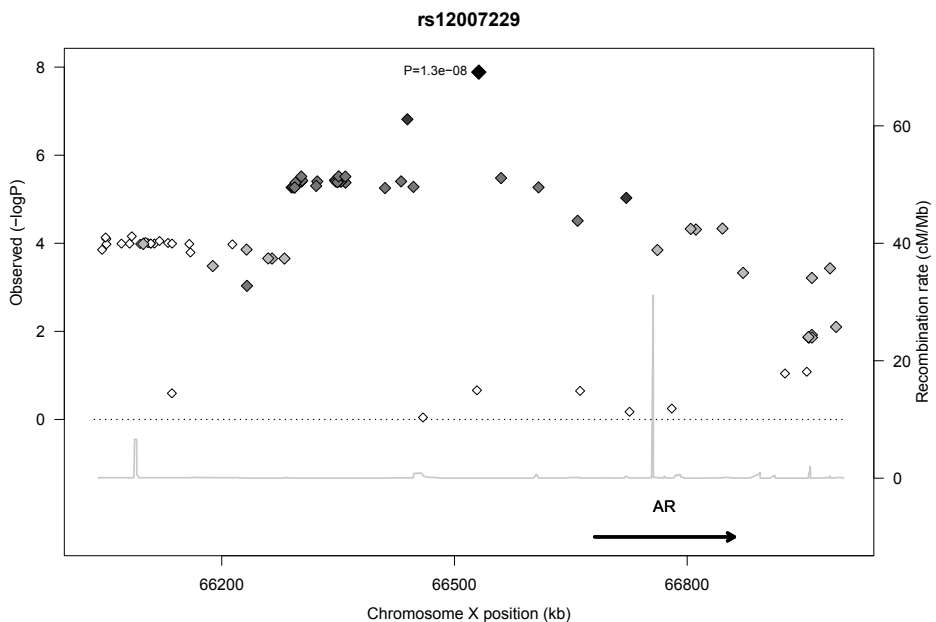


Figure 2. Regional association plot for the top hit rs12007229 on the X-chromosome. All SNPs (rectangles) are plotted with their p-values against their genomic position. Rectangles are size- and color-coded according to their linkage disequilibrium with the top SNP as follows: $r^2 < 0.2$ white; $0.2 < r^2 < 0.5$ light grey; $0.5 < r^2 < 0.8$ medium grey; $0.8 < r^2$ dark grey. Gene annotation is shown as the black arrow.

exons 2 and 3 the DNA-binding domain, and exons 4-8 the steroid-binding domain. The amino terminal domain contains two polymorphic trinucleotide repeats: a CAG repeat encoding for polyglutamine, and a GGN repeat encoding for polyglycine.¹² An expansion of the CAG repeat is responsible for a rare X-linked motor neuron disorder in men: spinal and bulbar muscular atrophy or Kennedy's disease.¹³ The main neurological manifestation of Kennedy's disease is a slowly progressive weakness and wasting of bulbar, facial, and limb muscles.¹⁴ Although cognitive impairment or dementia is not one of the main features of Kennedy's disease, several case reports and a family study suggest that cognitive dysfunction might be part of the disease's clinical manifestation.¹⁵

Although the *AR* gene has not been described in vascular dementia thus far, there are some reports on associations of the CAG repeat length within the normal range with cognitive function and Alzheimer disease.^{16,17} Whether the locus we found reflects an association of the *AR* gene, and possibly the CAG repeat length, with vascular dementia is unclear and needs further investigations. Another possibility is that this SNP reflects an association with the ectodysplasin A2 receptor gene (*EDA2R*), although the distance between rs12007229 and the *EDA2R* gene is larger than with the *AR* gene.¹⁸

Some methodological issues need to be addressed. Strengths of our study are the large sample size from both a population-based cohort and an independent clinic-based sample. The diagnosis of vascular dementia can be difficult, especially in the subtype of post-stroke vascular dementia. If a stroke is fatal or too severe (e.g. leading to severe aphasia or hemiplegia) the diagnosis of vascular dementia cannot be made leading to underestimation of the effect size. Another phenomenon we encounter in our study is the so-called "winner's curse", where the effect estimates in the discovery set are overestimated resulting in much greater effect sizes than in the replication analyses.¹⁹ Given our numbers and the MAF of 7%, we had >80% power to detect an OR of 3.0 in the discovery at alpha 5×10^{-8} , and >80% power to find an OR of 1.4 in the replication at alpha 0.05.²⁰

In conclusion, we report a new locus for vascular dementia on the X-chromosome close to the *AR* gene, which we replicated in an independent sample.

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7

Prediction of dementia

Chapter 7

Prediction of dementia

Elisabeth M.C. Schrijvers, Marielle M.F. Poels, Tom den Heijer, Peter J. Koudstaal,
Albert Hofman, Wiro J. Niessen, M. Arfan Ikram, Monique M.B. Breteler

ABSTRACT

Background Prediction models that can identify persons at high risk of developing disease are important for prevention and research. A risk factor of disease is not necessarily useful as a risk predictor, but the distinction between risk factors and risk predictors is often not made or properly evaluated. For dementia, several prediction scores have been proposed, but the additional value of the separate predictors is often not assessed. The aim of our study was to assess which risk factors of dementia have additive value in predicting the risk of developing dementia within ten years. Furthermore, we wanted to evaluate the additional value of brain imaging in prediction of dementia.

Methods We first assessed the predictive value of identified risk factors in participants from the Rotterdam Study, a large population-based cohort study in the Netherlands. Participants were free of dementia at baseline (1990-1993) and, because predictive factors may differ according to age, divided into two age groups; 60-75 years (n=3401) and 75-90 years (n=1391). Ten-year dementia risk prediction was assessed by the C-statistic. The model that gave the best prediction was then validated in participants from the Rotterdam Scan Study who were 60-90 years and free of dementia (n=486) at time of brain MRI scanning (1995-1996). We assessed the additional predictive value of brain volumes and small vessel disease on MRI above the basic prediction model.

Results After ten years of follow-up, 185 persons had developed dementia in the younger age group, 307 in the older age group, and 49 in the Rotterdam Scan Study. The best prediction was reached with a model of age, *APOE* genotype, MMSE score, and subjective memory complaints in both age groups, with a c-statistic of 0.731 in the younger and 0.599 in the older group. Vascular risk factors were not useful as predictors. The model was validated in the Rotterdam Scan Study with a c-statistic of 0.828, and further improved after adding total brain volume and hippocampal volume to the model (c-statistic 0.855). When we stratified the predicted risk in three categories (low, intermediate and high risk), the actual ten-year risk of dementia corresponded quite well with the predicted risk.

Conclusions Although many known risk factors of dementia are not useful as predictors of ten-year risk of dementia, a prediction based on only a few predictors may be useful in identifying persons with a high risk of developing dementia in the next decade.

INTRODUCTION

With an increasing absolute number of people who develop dementia worldwide¹ and the focus in research on finding therapeutic strategies that may slow down or stop progression of dementia, there is much interest in developing a tool that can identify individuals at a high risk of developing dementia in the near future. Such a predictive tool could help to focus clinical trials and preventive strategies on persons with a high risk of developing dementia.² Several risk scores for dementia both in mid-life and in late life have been proposed.³⁻⁶ These risk scores include factors such as demographics, life style factors, medical history, brain MRI findings and genotyping. However, the additional value of the separate predictors is often not assessed and especially the incremental value above age, which is currently still the most important risk factor of dementia, is not measured. Furthermore, the presence of an association between a risk factor and a disease does not necessarily imply that this risk factor will be useful for risk prediction. For use as a risk predictor, the association with disease has to be strong and there must be enough variation of the risk factor within the population.⁷ This raises the question whether the risk factors that have been included in previous risk scores really contribute to the risk prediction. The aim of our study was to assess which risk factors of dementia can be used as predictors of developing dementia within ten years and what these factors add to the most important risk factor age. Furthermore, we wanted to evaluate the difference in prediction based on predictors that are easily available in general practice (lifestyle factors, medical history etc.) with a prediction based on factors that include additional measurements like MRI or genotyping. We used data from the population-based Rotterdam Study and Rotterdam Scan Study to create a ten year dementia prediction model and evaluate the performance of the prediction.

METHODS

The Rotterdam Study and the Rotterdam Scan Study

The Rotterdam Study is a prospective population-based cohort study that is conducted among all inhabitants aged 55 years and older of Ommoord, a district of Rotterdam, the Netherlands.⁸ Of 10,274 eligible subjects, 7,983 (78%) participated in the baseline examinations between 1990-1993. 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.

In 1995-1996, a random subset of the Rotterdam Study was invited to undergo brain imaging. Details of the study have been described elsewhere.⁹

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

Study population

We first developed a predictive model using data from the baseline cohort of the Rotterdam Study. Because we wanted to validate this model in the Rotterdam Scan Study, in which the age range at baseline was 60-90 years, we excluded from this baseline cohort all participants younger than 60 years (n=1,230) or older than 90 years (n=265) as well as all persons who consented to participate in the Rotterdam Scan Study in 1995-1996. Furthermore, we excluded 386 participants who were not screened for dementia and 374 participants who were diagnosed with prevalent dementia at baseline, as well as 464 participants with missing data on Apolipoprotein E (*APOE*) genotype. This left us with 4,792 participants at risk of dementia to be included in the analyses.

We then evaluated the performance of the prediction model in participants from the Rotterdam Scan Study and assessed the additional predictive value of brain imaging. In the Rotterdam Scan Study, 486 participants had complete and reliable MRI data and were free of dementia at the time of MRI scanning.

Dementia case finding

Participants were screened for dementia at baseline and follow-up visits using a three-step protocol.¹⁰ Two brief tests of cognition (Mini-Mental State Examination (MMSE)¹¹ and Geriatric Mental State schedule (GMS)¹² organic level) were used to screen all participants. Screen-positives (MMSE score<26 or GMS organic level>0) underwent the Cambridge examination for mental disorders of the elderly.¹³ Participants 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 digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.¹⁰ The diagnosis of dementia was made in accordance with internationally accepted criteria (DSM-III-R)¹⁴ by a panel of a neurologist, neuropsychologist and research physician. The follow-up with regard to dementia diagnosis was virtually complete until January 1, 2007.

Assessment of risk factors

We included all factors that have been identified as risk factors of dementia that were available in the Rotterdam Study: age, sex, education, subjective memory complaints, smoking, diabetes, stroke, blood pressure, hypertension, obesity, *APOE* genotype, total cholesterol and HDL-cholesterol. Educational level was categorized into four groups: low education (primary education only), low-intermediate education (primary education with an unfinished higher education or lower vocational education), high-intermediate education (intermediate vocational education or general secondary education) and high education (higher vocational education or university). The presence of memory complaints was assessed by the question "Do you have memory complaints?" in the Rotterdam Study, and in the Rotterdam Scan Study by the question "Have there been moments in the last month that you felt forgetful?" Smoking habits were categorized as current, former and never cigarette smoking. Diabetes mellitus was considered present if participants reported use of antidiabetic medication or when the random or post-load serum glucose level was greater than 11.1 mmol/L. History of stroke at baseline was assessed during the baseline interview and verified by reviewing medical records.

Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in a sitting position. Hypertension was defined as a blood pressure $\geq 140/90$ or use of anti-hypertensive medication, prescribed for the indication of hypertension. Height and weight were measured at the research center and the body mass index (BMI) was calculated as a measure of obesity. *APOE* genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis.¹⁵ Non-fasting serum total cholesterol and HDL cholesterol concentrations were determined by an automated enzymatic procedure.

Brain imaging

Brain MRI was performed in the Rotterdam Scan Study on a 1.5-Tesla MRI System (VISION MR, Siemens AG) and included T1, proton-density and T2-scans.⁹ In addition, a high-resolution T1, inversion-recovery, 3-D HASTE sequence was acquired. Slice thickness was 5 mm for T1, T2, and proto-density sequences, and 1.25 mm for the HASTE sequence. Pre-processing steps, the segmentation algorithm, and validation results have been described previously.⁹ Lacunar infarcts were rated visually as focal hyperintensities on T2-images, ≥ 3 mm in size. In order to distinguish infarcts from cerebral white matter lesions (WML), lesions in the white matter also had to have corresponding prominent hypointensities on T1-weighted images. Proton-density

sequences were used to distinguish infarcts from dilated perivascular spaces.⁹ Cortical infarcts were those infarcts showing involvement of gray matter, and persons with both lacunar and cortical infarcts were included in the group with cortical infarcts.⁹ Left and right hippocampal volumes were segmented separately using an automated segmentation method as described in detail earlier.¹⁶ The mean volume of the left and right hippocampus was used in the analyses.

Statistical analyses

The follow-up time was calculated from the date of study entry or MRI date until the date of dementia diagnosis, death, or end of follow-up. Follow-up time after ten years was censored. Missing values in covariates ($\leq 4\%$) were imputed using missing value analysis in SPSS. For the first step, we divided the baseline cohort of the Rotterdam Study in two age groups; 60 to 75 years ($n=3401$) and 75-90 years ($n=1391$). We used Cox proportional hazards regression analysis to evaluate which possible risk factors were associated with the risk of incident dementia during ten years of follow-up. We adjusted for age and sex when applicable.

We then assessed the predictive value of each variable that had a p-value below 0.200 in the Cox model. The ten-year risk of dementia was estimated by using the Kaplan-Meier method. To examine how well each factor discriminates between people who will develop dementia and people who will not during ten years of follow-up, we computed the C-statistic for survival data.^{17,18}

First, we assessed the predictive performance of age alone over a 10-year follow-up period. We examined whether the predictive accuracy increased when information on the risk factors with a p-value < 0.200 were added to this prediction model. We then used a forward approach to create a basic prediction model including factors that improved the C-static until the C-statistic no longer improved. To minimize any over-optimism in our model, we assessed the internal validity of the models using the bootstrap re-sampling technique, generating 1000 bootstrap samples and using the average optimism to correct the predictive performance of the original models.¹⁹ Second, we validated the predictive performance of age and the improvement of prediction with the basic prediction model in the participants from the Rotterdam Scan Study. Because the number of cases in the Rotterdam Scan Study was limited, and the basic prediction models did not differ much in the two age groups, we did not divide the participants of the Rotterdam Scan Study in age groups, but assessed the prediction in the total group.

Third, we investigated the associations of brain volumes and infarcts on MRI with the risk of developing incident dementia with Cox proportional hazards models, adjusted for the variables from the basic prediction model. Brain volumes were ex-

pressed as percentage of the intracranial volume and WML volume was natural log transformed because of a non-normal distribution. We then added the variables with a p-value <0.200 to the basic prediction model and assessed the predictive accuracy by computing the C-statistic using the abovementioned forward approach. We used the bootstrap re-sampling technique as mentioned above, generating 200 bootstrap samples instead of 1000 because of a smaller number of participants.

Finally, we wanted to assess whether the final prediction model more accurately stratifies individuals into higher or lower risk categories than the predictive factors that are easily assessed in general clinical practice. We therefore created reclassification tables. We classified the participants into three risk categories (<10% or low, 10-25% or intermediate, and >25% or high). First, people were classified into these three categories on the basis of a model containing age, MMSE score and presence of subjective memory complaints. Second, we reclassified persons into these three categories on the basis of our final prediction model. Finally, we compared these categories and investigated the accuracy of these reclassifications using the Kaplan-Meier method to calculate the 10-year risk of dementia.

All analyses were performed using the statistical packages SPSS 15.0 for Windows (SPSS, Inc., Chicago, Illinois) and R version 2.12.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics of the Rotterdam Study and the Rotterdam Scan Study are shown in Table 1. After 30,327 person years, 185 persons had developed incident dementia in the Rotterdam Study in the age group 60-75, and in the age group 75-90, 307 persons had developed dementia after 8,667 person years. Table 2 shows the hazard ratios and corresponding 95% confidence intervals and p-values of all tested possible risk factors for incident dementia in the two age groups. Of the possible risk factors tested, age, *APOE* genotype, MMSE score and subjective memory complaints were strongly and significantly associated with the risk of developing incident dementia during ten years of follow-up in both age groups. In the younger age group HDL cholesterol was also significantly associated with dementia, and the associations of cigarette smoking, systolic blood pressure, total cholesterol, body mass index, hypertension, and stroke with the risk of dementia all had a non-significant p-value <0.200. In the older age group, diabetes was significantly associated with the risk of dementia, and cigarette smoking and stroke gave non-significant p-values <0.200. The predictive value of each of these risk factors and the combination of the factors that added value above a prediction based on age only is shown in Table 3. The C-statistic

Table 1. Baseline characteristics of the study population according to age group

		Age 60-75 years		Age 75-90 years	
		Rotterdam Study 1990-1993 N=3401	Rotterdam Scan Study 1995-1996 N=282	Rotterdam Study 1990-1993 N=1391	Rotterdam Scan Study 1995-1996 N=204
Age (years)		67.1 ± 4.2	67.6 ± 4.0	80.5 ± 3.9	81.3 ± 3.8
Female		1915 (56%)	146 (52%)	946 (68%)	99 (49%)
APOE ε4-alleles	0	2453 (72%)	186 (66%)	1026 (74%)	149 (73%)
	1	865 (25%)	86 (31%)	346 (25%)	52 (26%)
	2	83 (2%)	8 (3%)	19 (1%)	3 (2%)
Educational level	Low	643 (19%)	37 (13%)	570 (41%)	60 (29%)
	Low-intermediate	1109 (33%)	96 (34%)	370 (27%)	42 (21%)
	High-intermediate	1372 (40%)	120 (43%)	388 (28%)	84 (41%)
	High	277 (8%)	27 (10%)	63 (5%)	18 (9%)
MMSE (points)		27.8 ± 1.7	28.0 ± 1.9	26.8 ± 2.2	27.2 ± 2.4
Memory complaints		583 (17%)	71 (25%)	366 (26%)	75 (37%)
Cigarette smoking	Never	1074 (32%)	70 (25%)	742 (53%)	71 (35%)
	Past	1568 (46%)	153 (55%)	457 (33%)	110 (54%)
	Current	759 (22%)	57 (20%)	192 (14%)	23 (11%)
Systolic blood pressure (mmHg)		139 ± 21	143 ± 19	147 ± 23	151 ± 21
Total cholesterol (mmol/l)		6.70 ± 1.20	5.89 ± 1.00	6.40 ± 1.23	5.86 ± 1.13
HDL cholesterol (mmol/l)		1.35 ± 0.37	1.28 ± 0.36	1.33 ± 0.36	1.28 ± 0.37
Body mass index (kg/m ²)	<20	71 (2%)	5 (2%)	56 (4%)	10 (5%)
	20-25	1214 (36%)	105 (38%)	486 (35%)	67 (33%)
	25-30	1597 (47%)	136 (49%)	619 (45%)	95 (47%)
	>30	519 (15%)	34 (12%)	230 (17%)	31 (15%)
Hypertension (≥140/90)		1939 (57%)	185 (66%)	974 (70%)	171 (84%)
Diabetes		342 (10%)	11 (4%)	242 (17%)	27 (13%)
Stroke		64 (2%)	18 (6%)	69 (5%)	22 (11%)

Data are presented as mean ± standard deviation or numbers (percentages)

of age alone was 0.730 in the younger age group and 0.599 in the older group. *APOE* genotype, the presence of subjective memory complaints and the MMSE score clearly improved the C-statistic in both age groups. In the younger group systolic blood pressure and HDL cholesterol slightly improved the C-statistic of age alone and both gave a small improvement of the model that included age, *APOE* genotype, MMSE score and subjective memory complaints (C-statistic improved from 0.801 to 0.803). Adding both factors together, however, did not give any further improvement.

Table 2. Associations of possible risk factors with incident dementia during 10 years of follow-up in the Rotterdam Study

		60-75 years		75-90 years	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Age (per year)		1.24 (1.19-1.29)	<0.001	1.09 (1.06-1.12)	<0.001
Female versus male		1.00 (0.74-1.34)	0.989	1.04 (0.80-1.34)	0.777
APOE ε4-alleles	0	1.00 (ref)		1.00 (ref)	
	1	2.79 (2.06-3.79)		1.68 (1.32-2.13)	
	2	8.53 (5.24-13.9)	<0.001	3.09 (1.52-6.27)	<0.001
Educational level	Low	1.00 (ref)		1.00 (ref)	
	Low-intermediate	1.11 (0.75-1.64)		1.17 (0.89-1.54)	
	High-intermediate	1.07 (0.73-1.59)		0.93 (0.69-1.25)	
	High	0.54 (0.24-1.22)	0.523	1.26 (0.77-2.08)	0.844
Primary education only		1.06 (0.79-1.44)	0.693	1.05 (0.83-1.32)	
MMSE (per point)		0.85 (0.79-0.90)	<0.001	0.87 (0.83-0.92)	<0.001
Memory complaints		1.65 (1.19-2.30)	0.003	1.83 (1.45-2.31)	<0.001
Cigarette smoking	Never	1.00 (ref)		1.00 (ref)	
	Past	1.14 (0.79-1.64)		1.21 (0.92-1.57)	
	Current	1.43 (0.94-2.16)	0.097	1.36 (0.94-1.98)	0.068
Systolic blood pressure (per 10 mmHG)		1.07 (0.996-1.14)	0.066	0.98 (0.66-1.45)	0.431
Total cholesterol (per mmol/l)		0.89 (0.79-1.01)	0.078	1.00 (0.91-1.10)	0.991
HDL cholesterol (per mmol/l)		0.63 (0.40-0.99)	0.044	1.03 (0.75-1.43)	0.843
Body mass index (per kg/m ²)	<20	0.79 (0.29-2.17)		1.15 (0.60-2.19)	
	20-25	1.00 (ref)		1.00 (ref)	
	25-30	0.82 (0.60-1.12)		0.90 (0.70-1.16)	
	>30	0.72 (0.46-1.14)	0.162	0.87 (0.63-1.22)	0.279
Hypertension (>=140/90)		1.28 (0.94-1.73)	0.115	1.03 (0.81-1.32)	0.327
Diabetes		1.07 (0.67-1.70)	0.784	1.37 (1.03-1.82)	0.032
Stroke		2.03 (0.95-4.33)	0.068	1.40 (0.87-2.26)	0.168

Analyses are adjusted for age and sex, for categorical variables the p-trend is given

In the older age group diabetes slightly improved the C-statistic when compared to age alone, but had no additive value when added to a model that included age, *APOE* genotype, MMSE score and subjective memory complaints. The best prediction was reached when we included these four factors in the model resulting in a C-statistic of 0.665.

Because in both age groups age, *APOE* genotype, MMSE score and subjective memory complaints were the strongest predictors, and only in the younger group there was only a very modest improvement in prediction after adding HDL-cholesterol

Table 3. Discriminative ability of various 10-year dementia risk prediction models in the Rotterdam Study

Factors included in the model	60-75 years	75-90 years
	C-statistic (95% CI)	C-statistic (95% CI)
Age	0.731 (0.695-0.766)	0.599 (0.564-0.633)
Age+APOE	0.787 (0.755-0.818)	0.629 (0.596-0.662)
Age+memory complaints	0.735 (0.700-0.770)	0.625 (0.592-0.658)
Age+ MMSE	0.751 (0.717-0.785)	0.628 (0.596-0.661)
Age+smoking	0.731 (0.696-0.766)	0.599 (0.565-0.634)
Age+stroke	0.730 (0.695-0.765)	0.599 (0.564-0.634)
Age+SBP	0.732 (0.697-0.768)	NA
Age+cholesterol	0.730 (0.694-0.766)	NA
Age+HDL cholesterol	0.732 (0.697-0.767)	NA
Age+BMI	0.729 (0.695-0.765)	NA
Age+hypertension	0.731 (0.696-0.767)	NA
Age+diabetes	NA	0.601 (0.566-0.635)
Age+APOE+MMSE	0.799 (0.770-0.829)	0.651 (0.619-0.683)
Age+APOE+MMSE+complaints	0.801 (0.771-0.830)	0.665 (0.634-0.696)
Age+APOE+MMSE+complaints+Diabetes	NA	0.664 (0.633-0.695)
Age+APOE+MMSE+complaints+SBP	0.803 (0.774-0.832)	NA
Age+APOE+MMSE+complaints+SBP+HDL	0.803 (0.773-0.883)	NA

Table 4. Associations of MRI brain measures and risk of dementia in the Rotterdam Scan Study

	HR (95% CI)	p-value
Hippocampal volume (% of ICV)	0.16 (0.06-0.44)	<0.001
Total brain volume (% of ICV)	0.81 (0.71-0.92)	0.002
White matter lesions (% of ICV)	1.21 (0.91-1.63)	0.196
Cortical infarcts	1.92 (0.80-4.61)	0.144
Lacunair infarcts	0.90 (0.47-1.73)	0.747

Analyses are adjusted for the basic prediction model: age, APOE, MMSE and memory complaints
White matter lesions were natural log transformed because of a non-normal distribution

or systolic blood pressure to the model, we used the first four factors as the basic prediction model we wanted to validate in the Rotterdam Scan Study.

In the Rotterdam Scan Study after 3,875 person years, 49 persons had developed incident dementia. The basic prediction model including age, APOE genotype, subjective memory complaints and MMSE score had a C-statistic of 0.828 in this population, and showed a clear improvement in prediction compared to a prediction based on age only (C-statistic 0.766). Adding HDL-cholesterol or systolic blood pressure to the model gave no improvement of the C-statistic. The associations of

Table 5. Discriminative ability of adding brain volumes to the basic prediction model in the Rotterdam Scan Study (N=486)

Factors included in the model	C-statistic (95% CI)
Age	0.766 (0.707-0.825)
Basic model	0.828 (0.771-0.885)
Basic model + hippocampal volume	0.841 (0.779-0.904)
Basic model + Total brain volume	0.846 (0.797-0.905)
Basic model + White matter lesions	0.827 (0.769-0.884)
Basic model + Cortical infarcts	0.827 (0.771-0.882)
Basic model + Total brain volume+ hippocampal volume	0.855 (0.800-0.910)

Basic model=age, *APOE* genotype, MMSE and memory complaints

brain volumes, white matter lesions and cerebral infarcts with the risk of developing incident dementia during ten years of follow-up are shown in Table 4. Hippocampal volume and total brain volume were significantly associated with incident dementia, white matter lesions and cortical infarcts yielded p-values <0.200 and lacunar infarcts were not associated with the ten-year risk of dementia. White matter lesions and cortical infarcts did not improve prediction, but adding hippocampal volume and total brain volume to the basic prediction model improved dementia prediction with a C-statistic of 0.855 in the final model including age, *APOE* genotype, MMSE, subjective memory complaints, hippocampal volume and total brain volume (Table 5).

Table 6 shows the classification in risk categories based on a model with the easily measured factors from the final prediction model; age, MMSE score and subjective memory complaints and the reclassification after applying the final model that additionally included *APOE* genotype, hippocampal volume and total brain volume. The actual risk of dementia corresponded quite well with the predicted risks in the different risk strata in both prediction models. In total, 115 (24%) participants were reclassified into a different risk category after adding *APOE* genotype and brain volumes to the prediction model. This reclassification occurred mainly in the intermediate risk group, where 51 of 109 participants were reclassified into the low risk category and 20 participants were reclassified into the high risk category.

DISCUSSION

In this study we have assessed the predictive value of different risk factors of dementia and we showed that many known risk factors, especially vascular risk factors, are not useful as predictors to identify persons that have a high risk of developing dementia within ten years. The risk factors that are useful as predictors are age,

Table 6. Risk reclassification comparing the final prediction model to a model based on age, MMSE and memory complaints

Model based on age, MMSE and memory complaints	Prediction based on age, <i>APOE</i> genotype, MMSE, memory complaints, hippocampal volume and total brain volume				No. (%) Reclassified
	<10%	10-25%	>25%	Total	
< 10%					
No.	274	27	4	305	31 (10%)
Actual risk of dementia*, % (n)	2.8 (7)	13.0 (3)	62.5 (2)	4.4 (12)	
10-25%					
No.	51	38	20	109	71 (65%)
Actual risk of dementia, % (n)	2.6 (1)	13.7 (4)	49.4 (7)	14.1 (12)	
>25%					
No.	2	11	59	72	13 (18%)
Actual risk of dementia, % (n)	0 (0)	11.1 (1)	56.4 (24)	46.4 (25)	
Total					
No.	327	76	83	486	115 (24%)
Actual risk of dementia, % (n)	2.8 (8)	13.1 (8)	55.2 (33)	11.7 (49)	

* The actual risk of dementia was estimated based on Kaplan Meier method

MMSE score, presence of subjective memory complaints, *APOE* genotype, total brain volume and hippocampal volume.

Strengths of our study include the population based design, the virtually complete follow-up for incident dementia and the use of two different subpopulations from the same overall population that allowed us to validate the first part of the prediction model in an independent population. There are, however, some methodological issues that need to be addressed. First, some factors that have been previously associated with incident dementia were not significantly associated with incident dementia over ten years of follow-up in our current study. An explanation for this might be that associations can differ according to follow-up time and factors that have been associated with dementia during a short follow-up period of a few years are not necessarily associated with dementia during longer follow-up.²⁰ Next, because of limited numbers of cases in the Rotterdam Scan Study, we were not able to look at different dementia subtypes. Finally, there are several other variables that might be valuable in dementia prediction, including levels of β -Amyloid ($A\beta$) and tau in cerebrospinal fluid,²¹ $A\beta$ imaging,²² or more detailed information on ADL performance and daily activity²³. Because we do not have these data in our study, we were not able to assess whether these factors are of additional value to the prediction model. Our study shows a limited value of vascular factors in prediction of dementia during ten years of follow-up. This does not mean that vascular factors are not important in the etiology of dementia, but shows that an independent risk factor is not necessarily

useful as a risk predictor.⁷ Vascular risk factors are not very specific for dementia, whereas the factors that were useful as risk predictors are probably a better reflection of early brain pathology. It is not unexpected that MMSE score and presence of subjective memory complaints are part of the prediction model. Together with age, these are in fact important factors that a clinician will often use in his or hers first impression of cognitive status and likelihood to have or develop cognitive impairment or dementia. In addition, hippocampal volume and total brain volume can be seen as markers of brain pathology that is important in dementia.²⁴⁻²⁶ Altogether, our study shows a distinction between etiological risk factors that are not necessarily useful as risk predictors versus risk factors that can be seen as early markers of disease that are useful in risk prediction.

The C-statistic of a prediction based on age differed from 0.599 in the oldest age group to 0.766 in the Rotterdam Scan Study. This difference can be explained by the fact that in the Rotterdam Scan Study the variation in age was 30 years instead of 15. In addition, because the relative increase of dementia incidence is higher between age 60 to 75 than between age 75 to 90,²⁷ a higher predictive value of age in the younger group compared to the older group was expected.

Of the factors that we included in our final prediction model, three (age, MMSE score and presence of subjective memory complaints) are easily measured, while the other three factors require genotyping and brain imaging. Based on the easily measured factors it was already possible to classify persons into different risk categories that quite accurately reflect the absolute ten year risk of dementia. After adding information on *APOE* genotype, total brain volume and hippocampal volume to the prediction model 24% of the participants were reclassified into a different risk category. The majority of reclassification occurred in the intermediate category, where 65% of the persons were reclassified into either the low risk or the high risk group. The absolute risks in the reclassified groups corresponded well with the reclassification, indicating that the prediction did really improve. At present, there seems no direct use of dementia prediction in general clinical practice, because there is not yet a successful treatment available. Especially genotyping or a brain MRI will not be useful or feasible in the general population, when the results will not lead to any action. However, this prediction model could be useful in research that wants to focus on high and intermediate risk individuals. A two step approach could be used, with a first classification based on age, MMSE score and the presence of subjective memory complaints and additional brain imaging and *APOE* genotyping for the intermediate risk group to better classify their actual risk of dementia.

In conclusion, in the development of a prediction tool the additive value of a possible risk predictor should be established. A risk factor is not the same as a risk predictor and vascular risk factors of dementia do not seem useful in dementia risk predic-

tion. However, a prediction based on risk factors that are more a reflection of early brain pathology can be useful in identifying persons with a high risk of developing dementia.

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8

General discussion

The aim of the research described in this thesis was to search for non-invasive biomarkers and explore risk factors of dementia. In this chapter, I will summarize and review the main findings and discuss methodological issues and directions for future research.

MAIN FINDINGS

Incidence of dementia

Previous studies from the Rotterdam Study and other cohorts have shown that the incidence of dementia strongly increases with age with incidence rates of 0.5 per 100 person years at age 65 to 5 per 100 person years at age 90.¹ The prevalence of dementia shows a similar pattern with 1% of persons between 65 and 70 years old living with dementia and more than 40% of persons aged 90 years and older.² Because of an increasing life expectancy, the overall prevalence of dementia worldwide is increasing.³ Future projections of dementia prevalence typically assume stable incidence rates and do not take better prevention or treatment of vascular or other risk factors into account.^{4,5} It has been estimated that if interventions could delay the onset and progression of Alzheimer disease by a modest 1 year, there would be a significant reduction in the global burden of disease with nearly 9.2 million fewer cases of Alzheimer disease in 2050 than the projected number of 106.2 million cases.⁴ In chapter 2, we assessed the differences in dementia incidence rates, mortality rates and presence and treatment of vascular risk factors years between the subcohort of the Rotterdam Study that started in 1990 and the subcohort that started in 2000. All participants were between 60 and 90 years of age at study baseline. We found an overall reduction of 25% in dementia incidence rates in the 2000 subcohort compared to the 1990 subcohort. In strata of 10 year age groups and gender there was a consistent decrease in dementia incidence rates over time, except for men between 80 and 90 years old. Possibly due to a small number of cases in the 2000 subcohort our findings did not reach statistical significance. Evidence for a declining trend in dementia incidence was, however, further supported by the observation that when we compared both subcohorts, participants from the 2000 subcohort had on average less brain atrophy. They also had less cerebral small vessel disease, reflected by less WML volume and fewer lacunar infarcts. Furthermore, a recent paper also suggested a decrease in dementia incidence and a reduction of prevalence of cognitive impairment in the United States.⁶

Several factors might lead to a decline in dementia incidence, including better education,^{7,8} a decrease in stroke incidence⁹ and better prevention and treatment of vas-

cular risk factors.¹⁰ In our study we found that the 2000 cohort was better educated. Although the participants of the 2000 cohort actually had more vascular risk factors, they in parallel also used far more lipid lowering drugs and antithrombotics. Due to our sample size we could not determine a causal relation between these factors and the decrease in dementia incidence, but we hypothesized that especially the increase in use of medication for vascular risk factors might at least partly underlie the decrease in dementia incidence. Our findings suggest that the number of people living with dementia may rise less dramatically in the coming years than has previously been suggested, and that interventions on vascular risk factors may help reduce dementia incidence.

Biomarkers of dementia

In chapter 3.1 we have shown that an inappropriate selection of healthy controls can greatly influence the alleged performance of a biomarker panel. If the controls differ from the patients in other aspects than the studied disease, e.g. in age or general health status, and these differences are not taken into account when assessing the performance of a possible biomarker, the biomarker will reflect at least in part these other differences rather than the studied disease. For dementia one of the most important confounders is age, because the occurrence of dementia is strongly related to age. If controls are years younger than the patients, a biomarker might just reflect the age difference instead of the presence or absence of dementia. Often in small case-control studies where investigators assess differences between dementia patients and non-demented controls, there is no statistically significant difference in age or other possible confounding factors between the groups and investigators conclude that the groups are equal in age and other characteristics. Statistical significance is, however, greatly determined by sample size. The lack of a statistically significant difference should not be confused with proof of equality or with clinical relevance. An age difference of almost five years between patients and controls, as was the case in the discovery setting of the biomarker panel we used to illustrate our message,¹¹ is clinically quite relevant in dementia research.

In chapter 3.2, we evaluated the potential of plasma clusterin as a biomarker of the presence, severity, and risk of Alzheimer disease. Levels of plasma clusterin, also known as apolipoprotein J, were on average higher in Alzheimer patients than in controls and among Alzheimer disease patients, the average levels increased with disease severity. There was, however, no association between plasma clusterin levels and the development of Alzheimer disease over time, not even when we restricted the follow-up to three years to see whether plasma clusterin levels might have increased

due to subclinical disease. These association patterns were similar for all-cause dementia and vascular dementia. These results suggest that plasma clusterin is not a potential early biomarker of Alzheimer disease as was suggested in a recent study that found plasma clusterin to be associated with brain atrophy, baseline disease severity and rapid clinical progression of Alzheimer disease.¹² The association with severity of disease measured with the MMSE in that study was, however, consistent with the observations in our study. Furthermore, our results are in line with another report from the Rotterdam Scan Study, in which serum levels of clusterin measured by multiple reaction monitoring (MRM) assay were not different between 43 pre-symptomatic Alzheimer patients and 43 age- and gender matched controls.¹³

Risk factors of dementia

The retina provides a unique insight into the brain's microvasculature, because embryological, anatomical and physiological characteristics of the retinal vasculature are similar to the cerebral circulation and the retina is easy to visualize non-invasively.^{14,15} Retinal microvascular abnormalities can be seen as markers of cerebral microvascular disease. In chapter 4, we studied the associations between retinal microvascular abnormalities and the risk of dementia. Retinal vascular caliber was associated with the risk of vascular dementia during a mean follow-up of 11.6 years, but not with the risk of Alzheimer disease (chapter 4.1), whereas retinopathy at baseline was associated with prevalent all-cause dementia, Alzheimer disease and vascular dementia, but not with the risk of dementia or its subtypes during follow-up (chapter 4.2). The association between larger retinal venular caliber and vascular dementia might reflect cerebral hypoperfusion and subsequent ischemia.^{16,17} Because of large overlap between retinal caliber size between persons who will develop vascular dementia and persons who will remain free of dementia and because retinopathy does not occur before the clinical onset of dementia, there appears to be no use of retinal microvascular abnormalities as an early marker or risk predictor of dementia.

The relation between diabetes and dementia has been studied intensively, but the exact relation and mechanisms underlying a relation are still unclear.^{18,19} We investigated the associations of fasting levels of glucose, insulin and insulin resistance with the risk of developing Alzheimer disease and found that insulin and insulin resistance were associated with the risk of Alzheimer disease within three years of baseline, but not after three years (chapter 5.1). There was no association between glucose levels and the risk of Alzheimer disease. These results are in line with other studies with a long follow-up period that found no association of diabetes or the insulin metabolism and Alzheimer disease,^{20,21} and suggest that disturbances in the

insulin metabolism are not a causal factor in the development of Alzheimer disease. It is of interest that in chapter 7, in which we explore the use of several vascular factors as possible predictors of the 10 year risk of dementia, we found an association between diabetes and dementia in the participants aged 75 to 90 years but not in the participants aged 60 to 75 years. In contrast, in our study on glucose, insulin and insulin resistance we found no differences between age groups. Vascular risk factors that are related to dementia often show a stronger relation when measured at a younger age with relations diminishing, disappearing or even inverting at older age,²²⁻²⁵ so it was unexpected that we found the opposite for diabetes. We do not have a clear explanation for this finding; it does, however, add to the inconsistency in findings regarding the relation between diabetes or insulin metabolism and dementia, supporting our suggestion that the insulin metabolism is not a simple direct causal factor in the development of Alzheimer disease and dementia.

Another hormone that has been associated with dementia and Alzheimer disease is cortisol. Disturbances on cortisol release have been described in dementia and cognitive impairment. It is unknown whether these disturbances are a cause or consequence of disease. A relation between cortisol and dementia could either be causal, because higher levels of cortisol are associated with hippocampal atrophy, which can affect memory performance and lead to dementia,^{26,27} or reflect a consequence of dementia, because damage to the hippocampus may lead to reduced inhibition of the HPA axis and thus to higher serum cortisol levels.²⁸ We found no association between morning levels of serum cortisol and cognitive function, cognitive decline or the risk of developing dementia over time (chapter 5.2). These results suggest that morning levels of cortisol are not a causal factor in the development of cognitive impairment and dementia. These findings are in line with studies that found no increase of cortisol levels in MCI patients compared to controls, indicating that increased cortisol levels do not occur early in the development of Alzheimer's disease and might be the result, rather than the cause of the disease.^{29,30} A single measurement of serum cortisol does, however, not reflect all activity of the HPA-axis and we cannot answer the question how and to what extent the HPA-axis is involved in the pathogenesis of dementia.

In the genome-wide association study on vascular dementia described in chapter 6 we identified a new locus on the X-chromosome near the androgen receptor gene. We were able to replicate this locus in an independent sample. Thus far, vascular dementia has been underrepresented in studies unravelling the genetics of dementia. Our study is a first step in identifying new loci for vascular dementia. Given the fact that we know that most dementia cases are mixed,^{31,32} it is important to unravel the

genetics of dementia and the overlap and differences in genetics between different clinical subtypes. Therefore, the focus of genetic studies should not be limited to Alzheimer disease, but should also include vascular dementia, which is the second most common subtype both clinically,² and neuropathologically.^{31,32}

Prediction of dementia

In chapter 7, we have assessed which risk factors of dementia have additive value in predicting the risk of developing dementia within ten years. We found that vascular risk factors did not contribute in the risk prediction of dementia, neither in participants aged 60 to 75 years, nor in participants aged 75 to 90 years. The best prediction was reached with a model that included age, *APOE* genotype, MMSE score, subjective memory complaints, total brain volume and hippocampal volume. When we stratified the predicted risk into three categories (low, intermediate, and high risk), the actual ten-year risk of dementia corresponded quite well with the predicted risk. We also showed that the classification into risk categories improved when comparing the full model to a model that included the factors that are easily measured in clinical practice (age, MMSE score and presence of subjective memory complaints). Although currently, because of the lack of an effective treatment of dementia, there seems no direct use of dementia prediction in general clinical practice, our model could be used in research settings to identify persons at a high risk of developing dementia. Furthermore, our study shows that not all risk factors are useful as risk predictors and therefore should not be included in a prediction tool for dementia.

METHODOLOGICAL CONSIDERATIONS

Methodological issues specifically related to the different studies described in this thesis have been discussed in the respective chapters. Here I will discuss more general considerations that are important in cohort studies and dementia research.

Study design

All research described in this thesis is embedded in the Rotterdam Study, a large prospective population-based cohort study. The prospective design with measurements available years before a diagnosis of dementia is made enabled us to make inferences on causality and to explore at what time in the disease process a possible biomarker can be useful. However, because of the nature of dementia, which is a gradual process that may progress over many years before clinical symptoms occur

and interfere enough with daily functioning to make a diagnosis of dementia, even when a possible risk factor is measured years before the clinical diagnosis, one can never totally rule out the possibility of reversed causality. Furthermore, even when an association is found over a very long follow-up, like the association between retinal vascular caliber and vascular dementia described in chapter 4.1, it is not possible to determine the true nature of that association. For retinal vascular caliber it is not plausible that the retinal vessel itself is involved in the causal pathway of dementia, but we consider the changes in the retinal vessels a marker of other underlying processes. For other factors, the difference between a true causal relation or a common pathophysiology can be less clear.

Diagnosis of dementia

In the Rotterdam Study, follow-up for incident dementia is virtually complete until 1 January 2007, because we are able to continuously monitor all participants through linkage of the study database with medical records from general practitioners. Even when a participant does not attend the follow-up visits where an in-person screening is done, we are still able to determine whether he or she develops dementia. Of course, dementia can be difficult to detect in its early stage and especially if a person does not come to the follow-up visits anymore, we cannot rule out the possibility that we might have missed a few dementia cases.

A more important problem is the diagnosis of the subtype of dementia. We can only do this based on clinical criteria,^{33,34} while we know from neuropathological studies that most dementia patients have mixed brain pathology with combinations of Alzheimer disease pathology, vascular pathology and other pathology like Lewy bodies.^{31,32} This means that a diagnosis of Alzheimer disease represents a heterogeneous group of different underlying pathologies that have led to the dementia and this makes it more difficult to find risk factors and biomarkers that are specific for the disease. On the other hand, this heterogeneity of the clinical diagnosis is also one of the reasons we need biomarkers; namely to help in the differential diagnosis of different dementia subtypes.

Currently, efforts are being undertaken to adapt the existing diagnostic criteria for dementia and Alzheimer disease to the current state-of-the-art scientific knowledge.³⁵⁻⁴⁰ Although this is definitely an important step in the right direction and different workgroups have tried to include biomarkers in the new guidelines, the main clinical criteria do not differ that much from the old criteria and the role of biomarkers is still limited. Biomarkers that have been incorporated in new criteria include amyloid PET imaging, cerebrospinal fluid levels of A β and tau, fluorodeoxyglucose uptake on PET, and atrophy on structural magnetic resonance. These biomarkers are

not readily available in all clinical settings and, perhaps more importantly, there are currently no standardized guidelines on how to interpret the different biomarkers. More research is needed to define when a biomarker can be interpreted as clearly normal, clearly abnormal or in between. Furthermore, these guidelines still focus primarily on the diagnosis of Alzheimer disease and offer no clear solution for the distinction between 'pure' Alzheimer disease and mixed brain pathology. Even when biomarkers support a role of underlying Alzheimer pathology in a patient with dementia, this does not rule out the existence of another pathology in the brain, which contribution to the dementia might be equally important.

Another consideration in dementia research is the age of onset of dementia. We know that the patterns of risk factors change over time, and that especially vascular factors are associated with late-life dementia when measured at a younger age, but not at older ages.²²⁻²⁵ This suggests that the processes leading to dementia might also partially differ at different ages and that a person who is diagnosed with Alzheimer disease at age 70 may not be fully comparable to a person who is diagnosed with Alzheimer disease at age 90. This is also supported by neuropathological studies that have found that the relationship between neuropathological findings and dementia varies with age and that in the oldest old there is considerable overlap between the neuropathological features of Alzheimer disease in persons with or without dementia.^{41,42} Aging is, however, a difficult concept to truly quantify on an individual level.⁴³ Of course calendar age is easy to establish, but biological age is not. The risk factor patterns probably change very gradually over time from midlife to a very old age, but cut-off ages are difficult to define. It is, however, important to consider age differences in the search for new risk factors or biomarkers of dementia.

FUTURE DIRECTIONS AND CLINICAL IMPLICATIONS

The ultimate goal of dementia research is to unravel the etiology of the disease and find preventive and therapeutic treatments to help dementia patients and decrease the burden of disease both for individuals and on a population level. Future research should focus on the effect of treatment of vascular risk factors on the risk of developing dementia. The higher use of lipid-lowering drugs and antithrombotics we observed in the 2000 subcohort compared to the 1990 subcohort in combination with a decrease in dementia incidence, suggests that these treatments might help reduce dementia incidence. This is in line with other studies that reported associations of statins with a lower risk of dementia,⁴⁴⁻⁴⁶ and both antithrombotics and lipid-lowering drugs are preventive treatments for cerebrovascular disease.⁴⁷ Clinical trials with enough power that are focused on dementia as the primary outcome are needed to really

answer the question whether treatment of vascular risk factors reduces the incidence of dementia.¹⁰ At this point, there is no reason to treat vascular factors differently than according to current guidelines. However, there could be more awareness of the impact of vascular factors, especially in midlife, and the possible positive effect of treatment on dementia risk, as an extra drive for therapy compliance.

Although plasma clusterin does not seem useful as an early marker of Alzheimer disease, the association that we and others¹² have found with severity of disease is very interesting and deserves further investigation. This association suggests that plasma clusterin might be useful as a marker of progression of disease. It would be interesting to study the change in plasma clusterin levels over time in patients with Alzheimer disease and MCI. If plasma clusterin is indeed a marker of progression of disease, it might be useful in therapeutic trials as an extra tool to monitor disease progression and effect of treatment.

The search for biomarkers is a very challenging one. Currently, biomarker research is still mostly in the phase of focusing on a single marker or only a few markers at once, but for a multifactorial disease like dementia, it is more likely that combinations of biomarkers can have greater value. In the coming years research should shift towards investigating combinations and additive value of different biomarkers.

When exploring biomarkers as early diagnostic markers for dementia, the role of large population-based studies is crucial. Only in population-based cohort studies it is possible to avoid selection bias and investigate possible markers years before a person experiences cognitive complaints. It is, however, difficult in such large cohorts of participants from the general population to perform more invasive tests such as lumbar puncture or amyloid imaging on a large scale, and research that focuses on these methods may remain to be done primarily in clinical study settings for the years to come. However, the large number of participants in population-based cohorts will make it possible to search for combinations of different proteins to find a blood-based signature of dementia. Several studies have tried to find a blood-based signature of Alzheimer disease, and although thus far no such signature has been established, there is reason for optimism.⁴⁸ Currently, most of these studies still use a semi-focused approach and evaluate a large number of, often inflammatory, proteins. It will be very interesting to see what proteomic studies will bring us, when it can be done on a large scale. Comparable to what genome-wide associations studies have done in the field of genetics, plasma proteomics could be the next step for blood-based biomarkers. At this point in time, due to difficulties with the complexity of proteins, the huge dynamic range of different proteins, and the status of the technology, it is not yet feasible to perform a full proteomics study in a large cohort such as the Rotterdam Study. Hopefully, these problems will be overcome in the future. If that happens, equal to genome-wide association studies, we will need large

numbers of cases and controls to increase statistical power and to adjust for multiple testing, and population-based cohort studies will be an excellent platform to do this. In this thesis, I have made a first step in developing a prediction tool to identify people that have a high risk of developing dementia within ten years. Although we were able to explore the predictive value of many factors, there remain multiple other factors that deserve investigating. Simple measures that we lacked data on, such as grip strength or physical activity might have additive value in the prediction of dementia. Furthermore, more invasive methods such as CSF markers, amyloid imaging or other advanced imaging methods, and blood-based biomarkers also might be valuable prediction tools. It is important that studies investigating the predictive value of these factors, evaluate the additive value above at least age, but preferably also the other factors that we have shown to be predictive. Ultimately, these efforts can lead to the development of a strong prediction tool that can help identify persons at higher risk of dementia to specifically aim preventive and treatment strategies at these individuals, probably first in a research setting, but eventually also in clinical practice.

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9

Summary / Samenvatting

ENGLISH SUMMARY

Dementia is a devastating disease that is common in elderly people. Although many risk factors of dementia have been identified in the past decades, the exact mechanisms underlying the development of dementia are still unclear. When the clinical diagnosis of dementia is made, the actual neuropathological processes leading to dementia have already been ongoing for many years. Biomarkers that can detect these processes before a clinical diagnosis can be made are needed to identify persons who will develop dementia, to gain more insight in the pathogenesis of dementia and ultimately to help find new therapeutic agents that may alter or stop the disease. The aim of this thesis was to search for non-invasive biomarkers and explore risk factors of dementia. All research was embedded in the Rotterdam Study, a large prospective population-based cohort study among people of 55 years and older in Ommoord, a district of Rotterdam, the Netherlands.

In projections of future dementia prevalence, typically stable incidence rates are presumed, and a possible effect of changes in prevention and treatment of vascular risk factors is not taken into account. In **chapter 2** we assessed the differences in dementia incidence rates, mortality rates and presence and treatment of vascular risk factors between the subcohort of the Rotterdam Study that started in 1990 and the subcohort that started in 2000. We found a reduction in age-adjusted incidence rates of dementia and mortality rates in the 2000 subcohort compared to the 1990 subcohort. Evidence for a declining trend in dementia incidence was further supported by the observation that participants from the 2000 subcohort had on average less brain atrophy and cerebral small vessel disease on MRI. The prevalence of vascular risk factors increased between 1990 and 2000. This was paralleled by a strong increase in medication use for treatment of vascular risk factors. Our findings suggest that the number of people living with dementia may rise less dramatically in the coming years than has previously been suggested, and that interventions on vascular risk factors may help reduce dementia incidence.

In **chapter 3.1** we have shown that an inappropriate selection of healthy controls can greatly influence the alleged performance of a biomarker panel. If the controls differ from the patients in other aspects than the studied disease, e.g. in age or general health status, and these differences are not taken into account when assessing the performance of a possible biomarker, the biomarker will reflect at least in part these other differences and not the studied disease.

In **chapter 3.2**, we evaluated the potential of plasma clusterin as a biomarker of the presence, severity, and risk of Alzheimer disease. We found that levels of plasma clusterin, also known as apolipoprotein J, were on average higher in Alzheimer patients than in controls and among Alzheimer disease patients, the average levels

increased with disease severity. There was, however, no association between plasma clusterin levels and the development of Alzheimer disease over time, not even when we restricted the follow-up to three years to see whether plasma clusterin levels might have increased due to subclinical disease. These association patterns were similar for all-cause dementia and vascular dementia. These results show that plasma clusterin is not a potential early biomarker of Alzheimer disease, but it might be useful as a marker of disease progression.

In **chapter 4**, we studied the associations between retinal microvascular abnormalities and the risk of dementia. Retinal vascular caliber was associated with the risk of vascular dementia during a mean follow-up of 11.6 years, but not with the risk of Alzheimer disease (**chapter 4.1**). Retinopathy at baseline was associated with prevalent all-cause dementia, Alzheimer disease and vascular dementia, but not with the risk of dementia or its subtypes during follow-up (**chapter 4.2**).

Chapter 5 describes the associations of potential endocrine risk factors of dementia. In **chapter 5.1** we investigated the associations of fasting levels of glucose, insulin and insulin resistance with the risk of developing Alzheimer disease and found that insulin and insulin resistance were associated with the risk of Alzheimer disease within three years of baseline, but not after three years. There was no association between glucose levels and the risk of Alzheimer disease. Our findings suggest that the insulin metabolism influences the clinical manifestation of Alzheimer disease only within a short time-period and that disturbances in the insulin metabolism are not a simple direct causal factor in the development of Alzheimer disease. In **Chapter 5.2** we investigated the relation of morning levels of serum cortisol with cognition and dementia. We found no association between morning levels of serum cortisol and cognitive function, cognitive decline or the risk of developing dementia over time. These results suggest that morning levels of cortisol are not a causal factor in the development of cognitive impairment and dementia.

In the genome-wide association study on vascular dementia described in **chapter 6** we identified a new locus on the X-chromosome near the androgen receptor gene. We were able to replicate this locus in an independent sample.

In **chapter 7** we have assessed which risk factors of dementia have additive value in predicting the risk of developing dementia within ten years. We found that vascular risk factors did not contribute in the risk prediction of dementia, neither in participants aged 60 to 75 years, nor in participants aged 75 to 90 years. The best prediction was reached with a model that included age, *APOE* genotype, MMSE score, subjective memory complaints, total brain volume and hippocampal volume. Although currently, because of the lack of an effective treatment of dementia, there seems no direct use of dementia prediction in general clinical practice, our model could be used in research settings to identify persons at a high risk of developing dementia.

In **chapter 8**, I have discussed the main findings, methodological considerations and implications and suggestions for future research.

NEDERLANDSE SAMENVATTING

Dementie is een veel voorkomende aandoening bij ouderen. In de afgelopen decennia zijn vele risicofactoren van dementie ontdekt, maar de exacte mechanismen die leiden tot het ontstaan van dementie zijn nog niet bekend. Op het moment dat de diagnose van dementie klinisch wordt gesteld, zijn de onderliggende processen die leiden tot de dementie al jaren aan de gang. Om eerder in het ziektebeloop een diagnose te kunnen stellen zijn biomarkers nodig die deze onderliggende processen kunnen detecteren. Daarnaast kunnen biomarkers helpen meer inzicht te krijgen in de pathogenese van dementie en uiteindelijk ook bij het vinden van een behandeling die het ziekteproces kan vertragen of stoppen.

Het doel van dit proefschrift was het zoeken naar risicofactoren en niet-invasieve biomarkers van dementie. De verschillende studies maakten deel uit van het Erasmus Rotterdam Gezondheid Onderzoek (ERGO). Dit is een groot prospectief populatie cohort, waarbij mensen van 55 jaar en ouder uit Ommoord, een wijk in Rotterdam, sinds begin jaren '90 gevolgd worden.

Bij het voorspellen van de toekomstige prevalentie van dementie wordt er meestal vanuit gegaan dat de incidentie stabiel is gebleven en wordt geen rekening gehouden met een mogelijk effect van veranderingen in preventie en behandeling van vasculaire risicofactoren. In **hoofdstuk 2** hebben we de incidentie van dementie, de mortaliteitscijfers en de prevalentie en behandeling van vasculaire factoren vergeleken in het subcohort van ERGO dat werd gevolgd sinds 1990 met het subcohort dat werd gevolgd sinds 2000. We vonden dat de leeftijdsspecifieke incidentie van dementie en de mortaliteit lager waren in het 2000 subcohort in vergelijking met het 1990 subcohort. Bewijs voor een dalende trend in de incidentie van dementie werd verder ondersteund door de observatie dat deelnemers uit het 2000 subcohort minder hersenatrofie en cerebrale vaatschade hadden op MRI. Daarentegen was de prevalentie van vasculaire risicofactoren hoger in het 2000 subcohort dan in het 1990 subcohort. Dit ging echter wel gepaard met een nog sterkere stijging van medicatiegebruik voor vasculaire risicofactoren. Onze bevindingen suggereren dat het aantal mensen met dementie minder dramatisch zal stijgen dan in eerdere voorspellingen is weergegeven. Een betere behandeling van vasculaire risicofactoren helpt mogelijk bij het reduceren van de incidentie van dementie.

In **hoofdstuk 3.1** hebben we aangetoond dat een onjuiste selectie van een controlegroep ervoor kan zorgen dat een biomarker onterecht een goed onderscheid lijkt te maken tussen patiënten en controles. Als er niet wordt gecorrigeerd voor verschillen tussen de controlegroep en de patiënten, zoals leeftijd en algemene gezondheids-toestand, dan zal een biomarker op zijn minst voor een deel deze andere verschillen reflecteren en in plaats van de ziekte in kwestie.

In **hoofdstuk 3.2** beschrijven we de relatie tussen plasma clusterine, ook bekend als apolipoproteïne J, en dementie. Plasma clusterine spiegels waren hoger in mensen met de ziekte van Alzheimer dan in controles. Binnen de groep van Alzheimer patiënten was er een verband tussen hogere clusterine waarden en ernst van de dementie. Er was echter geen verband tussen clusterine en het ontstaan van Alzheimer gedurende de follow-up tijd. We vonden ook geen verband met het ontstaan van Alzheimer binnen drie jaar na bepaling van clusterine. De associaties van clusterine met dementie door alle oorzaken en vasculaire dementie vertoonden hetzelfde patroon. Deze resultaten laten zien dat plasma clusterine geen vroege marker is van de ziekte van Alzheimer, maar dat het mogelijk wel een marker kan zijn van progressie van de ziekte.

In **hoofdstuk 4** beschrijven we dat retinale vaatdiameters geassocieerd zijn met het risico op vasculaire dementie gedurende een gemiddelde follow-up van bijna twaalf jaar, maar niet met het risico op de ziekte van Alzheimer (**hoofdstuk 4.1**). Retinopathie was geassocieerd met prevalentie dementie, de ziekte van Alzheimer en vasculaire dementie, maar niet met het risico op het ontstaan van dementie of subtypes van dementie gedurende de follow-up (**hoofdstuk 4.2**).

Hoofdstuk 5 beschrijft de associaties tussen mogelijke endocriene risicofactoren en dementie. In **hoofdstuk 5.1** onderzochten we de associaties tussen het insuline-metabolisme en het risico op de ziekte van Alzheimer. We vonden dat insuline en insulineresistentie waren geassocieerd met het ontstaan van Alzheimer binnen drie jaar na meting, maar niet met het risico na drie jaar. We vonden geen verband tussen glucosewaarden en het risico op de ziekte van Alzheimer. Onze bevindingen suggereren dat het insulinemetabolisme alleen op korte termijn van invloed is op de klinische manifestaties van de ziekte van Alzheimer en dat er geen direct causaal verband is tussen verstoringen in het insulinemetabolisme en het ontstaan van dementie. In **hoofdstuk 5.2** onderzochten we het verband tussen ochtend serumwaarden van cortisol en cognitie en dementie. We vonden geen associatie tussen serum cortisol en cognitie, cognitieve achteruitgang of het risico op het ontstaan van dementie. Dit suggereert dat ochtend serumwaarden van cortisol niet causaal gerelateerd zijn aan het ontstaan van cognitieve stoornissen en dementie.

In de genome-wide associatie studie van vasculaire dementie in **hoofdstuk 6** vonden we een nieuwe locus op het X-chromosoom in de buurt van het androgeen receptor gen dat significant geassocieerd was met vasculaire dementie. We hebben deze bevinding kunnen repliceren in onafhankelijke data.

In **hoofdstuk 7** hebben we onderzocht welke risicofactoren van dementie bijdragen aan het voorspellen van het tienjaarsrisico van dementie. Vasculaire risicofactoren bleken niet bij te dragen aan de predictie van dementie in beide onderzochte leeftijdsgroepen van 60-75 jaar en 75-90 jaar. De meest optimale predictie werd bereikt

met een model bestaande uit leeftijd, *APOE* genotype, MMSE score, subjectieve geheugenklachten, totaal breinvolume en volume van de hippocampus. Omdat er nog geen effectieve behandeling van dementie voorhanden is, is een predictiemodel van dementie momenteel nog niet direct klinisch bruikbaar. Dit model kan echter wel gebruikt worden in een onderzoekssetting om mensen met een hoog risico op dementie te identificeren.

In **hoofdstuk 8** heb ik de implicaties van de belangrijkste bevindingen besproken en aandacht besteed aan methodologische overwegingen en suggesties voor toekomstig onderzoek.

10

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DANKWOORD

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LIST OF PUBLICATIONS

Manuscripts based on the studies described in this thesis

Chapter 2

Schrijvers EMC, Verhaaren BFJ, Koudstaal PJ, Hofman A, Ikram MA, Breteler MMB. Is dementia incidence declining? Trends in dementia incidence since 1990 in the Rotterdam Study. *Submitted*

Chapter 3.1

Schrijvers EMC, Hofman A, Koudstaal PJ, Breteler MMB. Biomarker profiles for Alzheimer disease: diagnosing disease or establishing health? *Submitted*

Chapter 3.2

Schrijvers EMC, Koudstaal PJ, Hofman A, Breteler MMB. Plasma clusterin and the risk of Alzheimer disease. *JAMA* 2011;305:1322-1326

Chapter 4.1

de Jong FJ*, **Schrijvers EMC***, Ikram MK, Koudstaal PJ, de Jong PTVM, Hofman A, Vingerling JR, Breteler MMB. Retinal vascular caliber and risk of dementia: The Rotterdam Study. *Neurology* 2011;76:816-821 (***equal contribution**)

Chapter 4.2

Schrijvers EMC, Buitendijk GHS, Ikram MK, Koudstaal PJ, Hofman A, Vingerling JR, Breteler MMB. Retinopathy and risk of dementia: The Rotterdam Study. *Submitted*

Chapter 5.1

Schrijvers EMC, Witteman JCM, Sijbrands EJ, Hofman A, Koudstaal PJ, Breteler MMB. Insulin metabolism and the risk of Alzheimer disease: The Rotterdam Study. *Neurology* 2010;75:1982-1987

Chapter 5.2

Schrijvers EMC, Direk N, Koudstaal PJ, Kirschbaum C, Hofman A, Tiemeijer H, Breteler MMB. Associations of serum cortisol with cognitive function and dementia: The Rotterdam Study. *Journal of Alzheimer's disease* 2011 Epub ahead of print

Chapter 6

Schrijvers EMC, Schürmann B, Koudstaal PJ, van den Bussche H, van Duijn CM, Hentschel F, Heun R, Hofman A, Jessen F, Kölsch H, Kornhuber J, Peters O,

Rivadeneira F, R  ther E, Uitterlinden AG, Riedel-Heller S, Dichgans M, Wiltfang J, Maier W, Breteler MMB, Ikram MA. Genome-wide association study of vascular dementia. *Submitted*

Chapter 7

Schrijvers EMC, Poels MMF, den Heijer T, Koudstaal PJ, Hofman A, Niessen WJ, Ikram MA, Breteler MMB. Prediction of dementia. *Submitted*.

Other Publications

Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, Bis JC, Smith AV, Carassquillo MM, Lambert JC, Harold D, **Schrijvers EMC**, Ramirez-Lorca R, Debette S, Longstreth WT, Jr., Janssens AC, Pankratz VS, Dartigues JF, Hollingworth P, Aspelund T, Hernandez I, Beiser A, Kuller LH, Koudstaal PJ, Dickson DW, Tzourio C, Abraham R, Antunez C, Du Y, Rotter JI, Aulchenko YS, Harris TB, Petersen RC, Berr C, Owen MJ, Lopez-Arrieta J, Varadarajan BN, Becker JT, Rivadeneira F, Nalls MA, Graff-Radford NR, Champion D, Auerbach S, Rice K, Hofman A, Jonsson PV, Schmidt H, Lathrop M, Mosley TH, Au R, Psaty BM, Uitterlinden AG, Farrer LA, Lumley T, Ruiz A, Williams J, Amouyel P, Younkin SG, Wolf PA, Launer LJ, Lopez OL, van Duijn CM, Breteler MMB. Genome-wide analysis of genetic loci associated with alzheimer disease. *JAMA* 2010;303:1832-1840

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PhD PORTFOLIO

Name: Elisabeth MC Schrijvers

Erasmus MC Department: Epidemiology

PhD period: 2008-2011

Research Schools: Nihes, COEUR

Promotors: Prof. dr. M.M.B. Breteler, Prof. dr. PJ Koudstaal

Research skills

2008-2010 MSc in Health Sciences, specialization Clinical Epidemiology, Netherlands Institute for Health Sciences (Nihes), Rotterdam, the Netherlands

2009 Biomedical English Writing and Communication

Oral Presentations

2011 'Risicofactoren en diagnose van dementie', Post-Academisch Onderwijs voor Huisartsen, Utrecht, the Netherlands

2011 'Plasma clusterin and the risk of Alzheimer disease: The Rotterdam Study', American Academy of Neurology 2011 Annual Meeting, Honolulu, USA

2010 'Leefstijl en andere risicofactoren bij dementie', Scientific session for supporters of the Internationale Stichting Alzheimer Onderzoek, Rotterdam, the Netherlands

2010 'Validating biomarker profiles for Alzheimer disease: diagnosing disease or establishing health?', FENS-IBRO European Neuroscience School: Neuroproteomics in animal model for neurodegenerative disorders, Smolenice, Slovakia

2009 'Prediction of Alzheimer's disease by means of a biomarker profile in the Rotterdam Study', International Conference on Alzheimer's disease 2009, Vienna, Austria

Poster Presentations

2011 'Trends in dementia incidence in the Rotterdam Study over the past 20 years', The 10th International Conference on Alzheimer's and Parkinson's Diseases, Barcelona, Spain

- 2009 'Insulin metabolism and the risk of Alzheimer's disease: The Rotterdam Study', International Conference on Alzheimer's disease 2009, Vienna, Austria

International Conferences

- 2011 American Academy of Neurology 2011 Annual Meeting, Honolulu, USA
- 2011 The 10th International Conference on Alzheimer's and Parkinson's Diseases, Barcelona, Spain
- 2010 FENS Forum of European Neuroscience 2010, Amsterdam, the Netherlands
- 2009 International Conference on Alzheimer's disease, Vienna, Austria

Seminars/Workshops/Courses

- 2010 FENS-IBRO European Neuroscience School: Neuroproteomics in animal model for neurodegenerative disorders, Smolenice, Slovakia
- 2010 NEURAD symposium: Update on Alzheimer's research: from fundamental mechanisms to therapeutic opportunities, Amsterdam, the Netherlands
- 2010 Biomarkers for risk prediction, Cardiovascular Research School Erasmus MC Rotterdam (COEUR), the Netherlands
- 2009 Peripheral and intracranial obstructive vascular disease, COEUR, Rotterdam, the Netherlands
- 2008 Research Institute for Diseases in the Elderly (RIDE) symposium, Amsterdam, the Netherlands
- 2008-2011 Research Seminars, department of Epidemiology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

Teaching

- 2008-2010 Teaching practicals in epidemiology to 4th year medical students, Rotterdam, the Netherlands

Other

- 2009-2011 Representative of PhD-students in the Rotterdam Study Management Team

- 2010-2011 Reviewing papers for various international scientific journals
- 2010 Organisation and chair of scientific session for supporters of the Internationale Stichting Alzheimer Onderzoek, Rotterdam, the Netherlands

ABOUT THE AUTHOR

Elisabeth Schrijvers was born on October 20, 1981 in Groningen, the Netherlands. After graduating in 1999 at the 'Praedinius Gymnasium' in Groningen, she started medical school at the Rijksuniversiteit Groningen. In 2004 she spent three months in Rome, Italy to learn the Italian language. In 2006 she participated in research on safety and clinical outcome after routine thrombolysis with tissue plasminogen activator in acute ischemic stroke patients aged 80 years and older at the department of Neurology at the University Medical Center Groningen (supervisor: dr. G.J. Luijckx). After obtaining her medical degree in 2007, she worked for one year as a resident in Neurology at the Martini Hospital in Groningen.

In January 2008 she moved to Rotterdam where she started the work described in this thesis at the department of Epidemiology of the Erasmus MC University Medical Center (head: Prof. dr. A. Hofman) under the supervision of Prof. dr. M.M.B. Breteler (department of Epidemiology) and Prof. dr. P.J. Koudstaal (department of Neurology). In August 2010 she obtained a Master of Health Sciences in Clinical Epidemiology at the Netherlands Institute for Health Sciences (Nihes). As of July 2011, she is working as a resident at the department of Neurology at Erasmus MC University Medical Center (head: Prof. dr. P.A.E. Sillevius Smitt).