Biomarkers and Risk Factors of Dementia

Elisabeth Marije Caroline Schrijvers
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Biomarkers and Risk Factors of Dementia

Biomarkers en risicofactoren van dementie

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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.\textsuperscript{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.\textsuperscript{2} Currently, dementia is still a clinical diagnosis of disturbances in cognitive functions that interfere with normal daily functioning.\textsuperscript{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.\textsuperscript{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.\textsuperscript{5,6} In addition, vascular risk factors have repeatedly been associated with not only vascular dementia, but also with Alzheimer disease,\textsuperscript{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 lead 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 (Aβ)-42 and tau that are altered in the cerebrospinal fluid of patients with Alzheimer disease early in the disease process.\textsuperscript{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.\textsuperscript{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
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.

REFERENCES

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. 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. Studies investigating trends in dementia incidence have reported different results. Some have reported no change in incidence rates. 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. A recent paper reported decreasing incidence rates between 1985 and 1994. 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. Since vascular risk factors increase the risk for dementia, 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. 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. 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.\textsuperscript{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.\textsuperscript{15} Two brief tests of cognition (Mini-Mental State Examination (MMSE)\textsuperscript{16} and Geriatric Mental State schedule (GMS)\textsuperscript{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.\textsuperscript{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.\textsuperscript{15} The diagnosis of dementia was made in accordance with internationally accepted criteria (DSM-III-R)\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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

<table>
<thead>
<tr>
<th>Subcohort</th>
<th>60-69</th>
<th>70-79</th>
<th>80-89</th>
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<tbody>
<tr>
<td></td>
<td>Men</td>
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<td>Men</td>
</tr>
<tr>
<td>Number</td>
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</tr>
<tr>
<td>1990</td>
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<td>1532</td>
<td>824</td>
</tr>
<tr>
<td>2000</td>
<td>553</td>
<td>636</td>
<td>194</td>
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<td>Age (years)</td>
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<tr>
<td>1990</td>
<td>64.9 ± 2.8</td>
<td>64.9 ± 2.9</td>
<td>74.4 ± 2.9</td>
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<td>2000</td>
<td>63.6 ± 2.8</td>
<td>63.1 ± 2.5</td>
<td>74.2 ± 2.8</td>
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<td>&lt;0.001</td>
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<tr>
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<tr>
<td>1990</td>
<td>243 (20%)</td>
<td>576 (38%)</td>
<td>267 (33%)</td>
</tr>
<tr>
<td>2000</td>
<td>86 (16%)</td>
<td>164 (27%)</td>
<td>36 (19%)</td>
</tr>
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<td>P-value</td>
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<tr>
<td>1990</td>
<td>138 ± 21</td>
<td>136 ± 21</td>
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<tr>
<td>2000</td>
<td>143 ± 21</td>
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<tr>
<td>1990</td>
<td>76 ± 11</td>
<td>74 ± 11</td>
<td>73 ± 12</td>
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<td>2000</td>
<td>81 ± 10</td>
<td>78 ± 10</td>
<td>79 ± 11</td>
</tr>
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<td>375 (31%)</td>
<td>504 (33%)</td>
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<td>185 (36%)</td>
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<tr>
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<td>322 (27%)</td>
<td>348 (23%)</td>
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<tr>
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<td>119 (23%)</td>
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<tr>
<td>1990</td>
<td>100 (8%)</td>
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<tr>
<td>2000</td>
<td>94 (18%)</td>
<td>227 (37%)</td>
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<td>1990</td>
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<td>126 (8%)</td>
<td>105 (13%)</td>
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<tr>
<td>2000</td>
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<td>58 (9%)</td>
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<td>0.52</td>
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Table 1. (continued)

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<td>2000</td>
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<tr>
<td>P-value</td>
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<td>Blood pressure lowering</td>
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<td>2000</td>
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<tr>
<td>P-value</td>
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<tr>
<td>Antidiabetic therapy</td>
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<td>Antithrombotics</td>
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<td>P-value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 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. |

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

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

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
The 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≤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).

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

<table>
<thead>
<tr>
<th>Age category</th>
<th>All</th>
<th>60-69</th>
<th>70-79</th>
<th>80-89</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of MRI</td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>Number</td>
<td>1995-1996</td>
<td>240</td>
<td>247</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>2005-2006</td>
<td>425</td>
<td>439</td>
<td>320</td>
</tr>
<tr>
<td>Mean (SE)</td>
<td>Intracranial volume (ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1995-1996</td>
<td>1204 (6.7)</td>
<td>1064 (5.7)</td>
<td>1204 (10.3)</td>
</tr>
<tr>
<td></td>
<td>2005-2006</td>
<td>1189 (4.9)</td>
<td>1051 (4.2)</td>
<td>1197 (5.4)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.10</td>
<td>0.09</td>
<td>0.52</td>
<td>0.49</td>
</tr>
<tr>
<td>Total brain volume (% of ICV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1995-1996</td>
<td>78.2 (0.2)</td>
<td>79.0 (0.2)</td>
<td>79.5 (0.3)</td>
</tr>
<tr>
<td></td>
<td>2005-2006</td>
<td>80.7 (0.1)</td>
<td>82.7 (0.1)</td>
<td>82.1 (0.2)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WML volume (% of ICV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1995-1996</td>
<td>0.83 (0.06)</td>
<td>1.34 (0.08)</td>
<td>0.57 (0.07)</td>
</tr>
<tr>
<td></td>
<td>2005-2006</td>
<td>0.68 (0.05)</td>
<td>0.79 (0.06)</td>
<td>0.44 (0.04)</td>
</tr>
<tr>
<td>P-value*</td>
<td>0.49</td>
<td>&lt;0.001</td>
<td>0.62</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

SE= standard error, WML= white matter lesions
* p-values based on analyses with WML natural log transformed
Means are adjusted for age, using ANOVA
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 α=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.\textsuperscript{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.\textsuperscript{11,12} In our study, however, apart from current smoking, vascular risk factors where 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 cohort\textsuperscript{28} and others,\textsuperscript{29,30} and both antithrombotics and lipid-lowering drugs are preventive treatments for cerebrovascular disease.\textsuperscript{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,\textsuperscript{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,\textsuperscript{33} which is an effect size not unlike many other effects seen in the cardiovascular field.\textsuperscript{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.\textsuperscript{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 healthcare 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.

REFERENCES


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
Chapter 3.2

Plasma clusterin and the risk of Alzheimer disease

Elisabeth M.C. Schrijvers, Peter J. Koudstaal, Albert Hofman, Monique M.B. Breteler
Chapter 3.2

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.\textsuperscript{1-3} The protein clusterin, also known as apolipoprotein J, has been suggested to be involved in the pathogenesis of Alzheimer disease.\textsuperscript{4,5} Clusterin has been found in the frontal cortex and hippocampus of postmortem Alzheimer disease brains\textsuperscript{6} and is increased in the cerebrospinal fluid of patients with Alzheimer disease.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{10} Two brief tests of cognition (Mini-Mental State Examination [MMSE]\textsuperscript{11} and Geriatric Mental State schedule (GMS)\textsuperscript{12} organic level) were used to screen all participants. Participants who screened positive (an MMSE score of <26 or GMS organic level of
Chapter 3.2

>0) underwent the Cambridge Examination for Mental Disorders of the Elderly. 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. The diagnoses of dementia and its subtypes were made in accordance with internationally accepted criteria for dementia (DSM-III-R), Alzheimer disease (NINCDS-ADRDA), and vascular dementia (NINDS-AIREN) 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. 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 inter assay 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 ε4 status was defined as carriership of 1 or 2 ε4 alleles. If APOE genotype was missing (n=42, 4.3%), APOE ε4 status was imputed as 0.28 (the proportion with an APOE ε4 allele in the total population with APOE genotyping). The MMSE score 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, the Word Fluency Test, and the abbreviated Stroop test. 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 ≤ 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 ε4 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. 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 subclinical 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>
<thead>
<tr>
<th>Table 1. Baseline characteristics of the study population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cohort at risk</strong></td>
</tr>
<tr>
<td>N=3709</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Women, No. (%)</td>
</tr>
<tr>
<td>APOE ε4-allele present, No. (%)</td>
</tr>
<tr>
<td>Education, only primary, No. (%)</td>
</tr>
<tr>
<td>Current smoking, No. (%)</td>
</tr>
<tr>
<td>Coronary heart disease, No. (%)</td>
</tr>
<tr>
<td>Diabetes, No. (%)</td>
</tr>
<tr>
<td>Hypertension, No. (%)</td>
</tr>
<tr>
<td>MMSE (points)</td>
</tr>
<tr>
<td>Clusterin (μg/ml)</td>
</tr>
</tbody>
</table>

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.
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 ε4 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 ε4 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

<table>
<thead>
<tr>
<th>Clusterin (μg/ml)</th>
<th>Prevalent Alzheimer disease at baseline n=60 OR (95% CI)(^1)</th>
<th>OR (95% CI)(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per SD</td>
<td>1.49 (1.12-1.98)</td>
<td>1.63 (1.21-2.20)</td>
</tr>
<tr>
<td>Per Quartile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47.2 -99.5</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>99.6-115</td>
<td>0.98 (0.38-2.45)</td>
<td>0.99 (0.36-2.78)</td>
</tr>
<tr>
<td>116-132</td>
<td>1.73 (0.70-4.25)</td>
<td>2.17 (0.83-5.68)</td>
</tr>
<tr>
<td>133-198</td>
<td>2.32 (1.03-5.26)</td>
<td>2.99 (1.25-7.16)</td>
</tr>
<tr>
<td>P-trend</td>
<td>0.02</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*OR=odds ratio, n=number of Alzheimer cases*
\(^1\) adjusted for age and sex
\(^2\) adjusted for age, sex, education, APOE ε4-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

<table>
<thead>
<tr>
<th>Clusterin (μg/ml)</th>
<th>Risk of incident Alzheimer disease during total follow-up n=208 HR (95% CI)(^1)</th>
<th>within three years n=76 HR (95% CI)(^1)</th>
<th>after three years N=132 HR (95% CI)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per SD</td>
<td>0.98 (0.84-1.15)</td>
<td>1.00 (0.85-1.17)</td>
<td>1.06 (0.82-1.37)</td>
</tr>
<tr>
<td>Per Quartile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47.2 -99.5</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>99.6-115</td>
<td>1.07 (0.68-1.68)</td>
<td>1.07 (0.67-1.71)</td>
<td>0.82 (0.39-1.71)</td>
</tr>
<tr>
<td>116-132</td>
<td>1.07 (0.69-1.68)</td>
<td>1.12 (0.70-1.77)</td>
<td>1.30 (0.66-2.55)</td>
</tr>
<tr>
<td>133-198</td>
<td>0.90 (0.57-1.43)</td>
<td>0.92 (0.58-1.46)</td>
<td>1.01 (0.48-2.11)</td>
</tr>
<tr>
<td>P-trend</td>
<td>0.66</td>
<td>0.77</td>
<td>0.68</td>
</tr>
</tbody>
</table>

*HR=hazard ratio, n=number of Alzheimer cases*
\(^1\) adjusted for age and sex
\(^2\) adjusted for age, sex, education, APOE ε4-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

<table>
<thead>
<tr>
<th>Odds of prevalent dementia</th>
<th>Risk of incident dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases OR (95% CI)(^1)</td>
<td>OR (95% CI)(^2)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>All-cause dementia</td>
<td>77 1.45 (1.13-1.85)</td>
</tr>
<tr>
<td>Vascular dementia</td>
<td>9  1.47 (0.77-2.83)</td>
</tr>
</tbody>
</table>

*OR=odds ratio, HR=hazard ratio*
\(^1\) adjusted for age and sex
\(^2\) adjusted for age, sex, education, APOE ε4-carriership, diabetes, smoking, coronary heart disease, and hypertension
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., 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

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

<table>
<thead>
<tr>
<th></th>
<th>Random subcohort</th>
<th>Prevalent AD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Difference (95% CI)</td>
</tr>
<tr>
<td><strong>MMSE (points)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>924</td>
<td>−0.004 (−0.128 to 0.120)</td>
</tr>
<tr>
<td>Model 2</td>
<td>924</td>
<td>−0.005 (−0.126 to 0.116)</td>
</tr>
<tr>
<td><strong>LDST (points)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>904</td>
<td>−0.010 (−0.437 to 0.417)</td>
</tr>
<tr>
<td>Model 2</td>
<td>904</td>
<td>0.002 (−0.407 to 0.411)</td>
</tr>
<tr>
<td><strong>WFT (points)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>916</td>
<td>0.22 (−0.13 to 0.58)</td>
</tr>
<tr>
<td>Model 2</td>
<td>916</td>
<td>0.22 (−0.13 to 0.58)</td>
</tr>
<tr>
<td><strong>Stroop trial 3 (seconds)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>898</td>
<td>0.38 (−0.89 to 1.65)</td>
</tr>
<tr>
<td>Model 2</td>
<td>898</td>
<td>0.35 (−0.90 to 1.59)</td>
</tr>
</tbody>
</table>

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 ε4-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., 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, but fits the hypothesis that the increased expression of clusterin in Alzheimer disease reflects a neuroprotective response. 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 formation through binding amyloid-beta or enhancing its clearance over the blood-brain barrier, clearance by endocytosis of amyloid-beta aggregates and cell debris to brain phagocytes, and inhibition of complement activation. The neurodegenerative changes that occur in Alzheimer disease may trigger an increased expression of clusterin. 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.

REFERENCES


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. 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. Moreover, pathological changes in the retinal microcirculation have been shown in patients with cerebrovascular disease, suggesting that retinal vessels may reflect concomitant cerebral microangiopathy.

During the late 1990s, a semi-automated system became available to reliably quantify retinal arteriolar and venular calibers. Several studies have shown that smaller arteriolar calibers were strongly related to higher blood pressure, whereas larger venular calibers were consistently associated with higher levels of inflammation markers, cholesterol, and both subclinical and clinical atherosclerosis. Furthermore, larger venular calibers were associated with an increased risk of stroke and progression of cerebral small vessel disease. 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. 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.\(^1\) First, participants were cognitively screened with the Mini-Mental State Examination (MMSE)\(^2\) and the Geriatric Mental State schedule (GMS)\(^3\) 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)\(^4\) 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)\(^5\), Alzheimer disease (NINCDS-ADRDA)\(^6\) and vascular dementia (NINDS-AIREN criteria).\(^7\) 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\(^9\) 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).\(^8\)
The rationale and procedures to measure and summarize retinal vascular calibers have been described.\textsuperscript{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 transparancies 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).

\textbf{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 (Immage\textsuperscript{®} 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 $\geq 11.1$ 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 (\textit{APOE}) genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis.\textsuperscript{26} \textit{APOE} $\varepsilon 4$-carriehership was defined as the presence of at least one \textit{APOE} $\varepsilon 4$-allele.

\textbf{Statistical analysis}

Analysis of covariance (ANCOVA), adjusted for age and sex, was used to compare baseline characteristics of participants with and without gradable fundus transparancies. 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. 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 ε4-allele is an important risk factor for Alzheimer disease, and may modulate the effects of vascular disease on the brain, we also performed the analyses within strata of APOE genotype (carriers vs. non-carriers of the ε4-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 transparancies are shown in table 1. Adjusted mean differences show that those with ungradable fundus transparancies 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 μm (SD: 14.4 μm; range 92.2-235.7 μm), and the mean summated venular caliber 222.2 μm (SD: 20.8 μm; range 135.1-313.6 μm). 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-
Retinal vascular caliber and dementia

The association 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

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics of the study population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gradable</strong>*</td>
</tr>
<tr>
<td><strong>N=5553</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Women (%)</td>
</tr>
<tr>
<td>Institutionalized (%)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
</tr>
<tr>
<td>High-sensitive C-reactive protein (mg/L)</td>
</tr>
<tr>
<td>Current smoking (%)</td>
</tr>
<tr>
<td>Diabetes (%)</td>
</tr>
<tr>
<td>Stroke at baseline (%)</td>
</tr>
<tr>
<td>Coronary heart disease (%)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>Use of antihypertensive medication (%)</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Table 2. Risk of dementia, Alzheimer disease and vascular dementia according to retinal vascular calibers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dementia</strong> without CVD</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td><strong>HR (95% CI)</strong></td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Arteriolar caliber</td>
</tr>
<tr>
<td>Per SD (14.4 μm) decrease, model 1</td>
</tr>
<tr>
<td>Per SD (14.4 μm) decrease, model 2</td>
</tr>
<tr>
<td>Per SD (14.4 μm) decrease, model 3</td>
</tr>
<tr>
<td>Venular caliber</td>
</tr>
<tr>
<td>Per SD (20.8 μm) increase, model 1</td>
</tr>
<tr>
<td>Per SD (20.8 μm) increase, model 2</td>
</tr>
<tr>
<td>Per SD (20.8 μm) increase, model 3</td>
</tr>
</tbody>
</table>

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
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 ε4-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. 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. 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, 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. Retinal venular dilatation is observed in the early stages of diabetic and venous stasis retinopathy, both of which are characterized by retinal ischemia. In turn, retinal ischemia has been related to lower cerebral blood flow. 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. 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. 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. 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, 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. 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.

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 transparancy 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.

REFERENCES


Chapter 4.1


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
Chapter 4.2

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 infarcts\(^10\) and ventricular enlargement\(^11\) over a 10 year period; features that also have been associated with dementia\(^12\) 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. Two brief tests of cognition (Mini-Mental State Examination (MMSE) and Geriatric Mental State schedule (GMS) 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). 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. The diagnoses of dementia and its subtypes were made in accordance with internationally accepted criteria for dementia (DSM-III-R), Alzheimer disease (NINCDS-ADRDA), and vascular dementia (NINDS-AIREN) 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 ≥ 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 (Immage® 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. ApoE ε4-carriership was defined as the presence of at least one APOE ε4-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 ε4-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>
<thead>
<tr>
<th>Table 1. Baseline characteristics of the study population</th>
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<tr>
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<tr>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Female (%)</td>
</tr>
<tr>
<td>Only primary education (%)</td>
</tr>
<tr>
<td>Carrier of APOE e4-allele (%)</td>
</tr>
<tr>
<td>Current cigarette smoking (%)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>Use of anti hypertensive medication (%)</td>
</tr>
<tr>
<td>Hypertension (&gt;=140/90)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Diabetes (%)</td>
</tr>
<tr>
<td>Stroke (%)</td>
</tr>
<tr>
<td>Coronary heart disease (%)</td>
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<tr>
<td>Retinopathy (%)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation or percentages
Retinopathy and dementia

Table 2. Odds ratios of retinopathy and prevalent dementia

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases/N</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>All dementia</td>
<td>No retinopathy</td>
<td>160/5800</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td></td>
<td>Retinopathy</td>
<td>34/473</td>
<td>2.04 (1.34-3.09)</td>
</tr>
<tr>
<td>Alzheimer disease</td>
<td>No retinopathy</td>
<td>124/5764</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td></td>
<td>Retinopathy</td>
<td>25/463</td>
<td>1.80 (1.11-2.91)</td>
</tr>
<tr>
<td>Vascular dementia</td>
<td>No retinopathy</td>
<td>22/5662</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td></td>
<td>Retinopathy</td>
<td>7/445</td>
<td>3.01 (1.26-7.21)</td>
</tr>
</tbody>
</table>

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

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-
ence of retinopathy might be used as a 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 diabetes\(^5\), 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,

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

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

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
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, while prospectively they only found an association with psychomotor speed and executive functioning, and not with memory. 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. 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 and we had previously found an association of retinal vascular caliber with incident vascular dementia, 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, retinopathy might be more a reflection of arteriolar damage from hypertension and diabetes. 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.
REFERENCES


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,
Chapter 5.1

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* ε4-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, 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. 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.

We previously reported from the Rotterdam study, a population based cohort study, an association between diabetes and a higher risk of Alzheimer disease. 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. 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. 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. 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). 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, which is the golden standard for measuring insulin resistance.

Dementia case finding

The diagnosis of dementia was made following a three-step protocol. Two brief tests of cognition (Mini-Mental State Examination (MMSE) and Geriatric Mental State schedule (GMS) organic level) were used to screen all participants. Screen-positives (MMSE score < 26 or GMS organic level > 0) underwent the Cambridge examination for mental disorders of the elderly (Camdex). 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. The diagnoses of dementia and Alzheimer disease were made in accordance with internationally accepted criteria for dementia (DSM-III-R) and Alzheimer disease (NINCDS-ADRDA) 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).

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 ε4-status was defined as carriership of one or two ε4-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 log₂-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 ε4-status, and the components of the metabolic syndrome (waist, systolic and diastolic blood pressure, triglycerides and HDL-cholesterol). 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 ε4-carriership. To test for interaction of APOE ε4-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 ε4-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. We used the relative risk due to interaction (RERI) to test for interaction on an additive scale. 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
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 1. Baseline characteristics of the study population

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

Data are presented as mean ± standard deviation or percentages
* Median (interquartile range) are presented because of a skewed distribution

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

<table>
<thead>
<tr>
<th></th>
<th>Short follow-up (max 3 years)</th>
<th>Medium follow-up (3-5.5 years)</th>
<th>Long follow-up (5.5-9.7 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=3139</td>
<td>N=2881</td>
<td>N=2566</td>
</tr>
<tr>
<td>Glucose (per SD)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>1.08 (0.86-1.35)</td>
<td>0.79 (0.58-1.08)</td>
<td>0.70 (0.51-0.96)</td>
</tr>
<tr>
<td></td>
<td>1.16 (0.93-1.45)</td>
<td>0.85 (0.61-1.18)</td>
<td>0.61 (0.41-0.89)</td>
</tr>
<tr>
<td>Insulin (per log_2)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>1.12 (0.83-1.51)</td>
<td>0.65 (0.48-0.89)</td>
<td>0.83 (0.60-1.14)</td>
</tr>
<tr>
<td></td>
<td>1.41 (1.02-1.96)</td>
<td>0.71 (0.49-1.02)</td>
<td>0.74 (0.51-1.08)</td>
</tr>
<tr>
<td>Insulin resistance (HOMA per log_2)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>1.12 (0.85-1.46)</td>
<td>0.68 (0.51-0.89)</td>
<td>0.80 (0.60-1.07)</td>
</tr>
<tr>
<td></td>
<td>1.39 (1.04-1.86)</td>
<td>0.72 (0.52-1.01)</td>
<td>0.70 (0.50-0.99)</td>
</tr>
</tbody>
</table>

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
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 ε4-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 ε4-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 ε4-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 ε4-status and glucose levels, insulin levels and insulin resistance on an additive scale, and a borderline significant interaction of APOE ε4-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 ε4-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.

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 ε4-carriership, with p-values for interaction

<table>
<thead>
<tr>
<th></th>
<th>APOE ε4 carriers</th>
<th>APOE ε4 non-carriers</th>
<th>P-values for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>multiplicative</td>
</tr>
<tr>
<td>Glucose (per SD)</td>
<td>0.91 (0.49-1.67)</td>
<td>1.25 (1.03-1.52)</td>
<td>0.165</td>
</tr>
<tr>
<td>Insulin (per log₂)</td>
<td>1.16 (0.65-2.06)</td>
<td>1.75 (1.16-2.64)</td>
<td>0.087</td>
</tr>
<tr>
<td>Insulin resistance (HOMA per log₂)</td>
<td>1.10 (0.66-1.84)</td>
<td>1.74 (1.21-2.51)</td>
<td>0.050</td>
</tr>
</tbody>
</table>

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

Different studies report conflicting results on the influence of the APOE ε4-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 ε4-allele, although none of these studies report if they have tested for additive interaction. The Uppsala Longitudinal Study of Adult Men showed a stronger effect of a low acute or early insulin response, assessed at midlife and at age 71 on the risk of Alzheimer disease in non-carriers of the APOE ε4-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 ε4-allele, which is conflicting with our results.

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 ε4-allele. Investigators from the Kungsholmen Project initially reported an effect of diabetes on the risk of Alzheimer disease only in carriers of the APOE ε4-allele, 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. These discrepant results fit our finding of no interaction between APOE ε4-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 ε4-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.

REFERENCES


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
ABS tr AC t

Chapter 5.2

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 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.\(^5\,^6\) 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. Two brief tests of cognition (Mini-Mental State Examination (MMSE) and Geriatric Mental State schedule (GMS) 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). 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. The diagnoses of dementia and Alzheimer disease were made in accordance with internationally accepted criteria for dementia (DSM-III-R) and Alzheimer disease (NINCDS-ADRDA), 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)\textsuperscript{28}, the Word Fluency Test (WFT)\textsuperscript{29} and the abbreviated Stroop test.\textsuperscript{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. \textit{APOE} ε4-status was defined as carriernesship of one or two ε4-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.\textsuperscript{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 \textit{APOE} ε4-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 ε4-carriership and we tested for interaction of APOE ε4-carriership by adding APOE ε4-status and the product of APOE ε4-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-
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 \textit{APOE} ε4-allele (p-values for interaction>0.09).

### Table 1. Baseline characteristics of the study population

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

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Difference 95% CI</th>
<th>Difference 95% CI</th>
<th>Difference 95% CI</th>
<th>Difference 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE (points)</td>
<td>-0.021</td>
<td>-0.084; 0.042</td>
<td>-0.049</td>
<td>-0.265; 0.167</td>
</tr>
<tr>
<td>LDST (points)</td>
<td>-0.049</td>
<td>-0.265; 0.167</td>
<td>0.047</td>
<td>-0.130; 0.223</td>
</tr>
<tr>
<td>Word fluency (points)</td>
<td>0.066</td>
<td>-0.612; 0.744</td>
<td>0.086</td>
<td>-0.626; 0.798</td>
</tr>
<tr>
<td>Stroop trial 3 (seconds)</td>
<td>-0.079</td>
<td>-0.301; 0.143</td>
<td>-0.111</td>
<td>0.262</td>
</tr>
</tbody>
</table>

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
Chapter 5.2

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

<table>
<thead>
<tr>
<th></th>
<th>MMSE (points)</th>
<th>LDST (points)</th>
<th>Word fluency (points)</th>
<th>Stroop trial 3 (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=2512</td>
<td>N=2344</td>
<td>N=2420</td>
<td>N=2202</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>0.012</td>
<td>0.026</td>
<td>0.018</td>
<td>0.131</td>
</tr>
<tr>
<td>95% CI</td>
<td>−0.031; 0.007</td>
<td>0.010; 0.062</td>
<td>−0.024; 0.061</td>
<td>−0.036; 0.298</td>
</tr>
<tr>
<td>Difference</td>
<td>0.010</td>
<td>0.018</td>
<td>0.002</td>
<td>0.105</td>
</tr>
<tr>
<td>95% CI</td>
<td>−0.031; 0.011</td>
<td>−0.021; 0.057</td>
<td>−0.044; 0.048</td>
<td>−0.075; 0.285</td>
</tr>
<tr>
<td>Difference</td>
<td>0.007</td>
<td>0.016</td>
<td>0.011</td>
<td>0.128</td>
</tr>
<tr>
<td>95% CI</td>
<td>−0.027; 0.013</td>
<td>−0.022; 0.054</td>
<td>−0.030; 0.062</td>
<td>−0.047; 0.303</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th></th>
<th>Total follow-up</th>
<th>Within 4.8 years</th>
<th>After 4.8 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dementia</td>
<td>AD</td>
<td>Dementia</td>
</tr>
<tr>
<td>HR 95% CI</td>
<td>n=243</td>
<td>n=210</td>
<td>n=122</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.01</td>
<td>0.89-1.15</td>
<td>1.00</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>0.87-1.15</td>
<td>0.99</td>
</tr>
</tbody>
</table>

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. 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. 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. 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. 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. 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 ε4-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 ε4-carriers, but not in non-carriers. Other longitudinal studies reported associations of higher levels of cortisol and more cognitive decline in Alzheimer patients, a slower decline in MCI patients and no association in normal controls. 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. 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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
Cortisol, cognition, and dementia

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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Genome-wide association study of vascular dementia
Chapter 6

Genome-wide association study of vascular dementia

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Chapter 6

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; \(p=1.3\times10^{-8}\)). 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. 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. 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 disease or focused on rare subtypes of vascular dementia like CADASIL. Recently, several genome-wide association studies (GWAS) have been done on Alzheimer disease, leading to the discovery of new loci. 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. 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. In addition, the cohort was continuously monitored for incident dementia through medical records from general practitioners. 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). 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.
Chapter 6

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. 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*10⁻⁸) and rs10491487 on chromosome 5 (p=3.7*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. Exon 1 of the AR-gene encodes the amino terminal domain,
### Table 2. Association of rs12007229 and vascular dementia in the different populations

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

MAF= minor allele frequency

**Figure 2.** Regional association plot for the tophit 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: r²<0.2 white; 0.2<r²<0.5 light grey; 0.5<r²<0.8 medium grey; 0.8<r² 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. 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. The main neurological manifestation of Kennedy’s disease is a slowly progressive weakness and wasting of bulbar, facial, and limb muscles. 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.

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. 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.

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. 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.

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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GWAS of vascular dementia


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
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 that 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 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. 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 statistical 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, 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 inversing at older age, 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 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. 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. 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 extend 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, 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,\textsuperscript{2} and neuropathologically.\textsuperscript{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, \textit{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, 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. 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. 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 Aβ and tau, fluorodeoxyglucose uptake on PET, and atrophy on structural magnetic resonance. These biomarkers are
General discussion

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. 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 others 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. 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 individu-
als, probably first in a research setting, but eventually also in clinical practice.

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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.
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. 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 gezondheidsstoestand, 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 apoliproteïne 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 prevalent 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 insuline-metabolisme 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 insuline-metabolisme 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.
Dankwoord

List of publications

PhD Portfolio

About the author
DANKWOORD

Op deze plaats wil ik graag iedereen bedanken die betrokken is geweest bij het tot stand komen van dit proefschrift.

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LIST OF PUBLICATIONS

Manuscripts based on the studies described in this thesis

Chapter 2

Chapter 3.1
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Chapter 3.2
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Chapter 4.1

Chapter 4.2

Chapter 5.1

Chapter 5.2

Chapter 6

Chapter 7

Other Publications


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PhD PORTFOLIO

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Oral Presentations

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

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
Chapter 10


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. Sillevis Smitt).