Genetic and Vascular Risk Factors for Cognitive Decline and Cerebral Small-Vessel Disease

Christiane Reitz

Acknowledgements

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Genetic and Vascular Risk Factors for Cognitive Decline and Cerebral Small-Vessel Disease

Genetische en vasculaire risicofactoren voor cognitieve achteruitgang en cerebrale microangiopathie

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	Prof.dr. C.M. van Duijn
	Prof.dr. R.P. Mayeux

For my family

Every man's memory is his private literature

Aldous Huxley
(1894-1963)

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Reitz C, Luchsinger JA, Tang MX, Manly J, Mayeux R. Impact of Plasma Lipids and Time on Memory Performance in Healthy Elderly without Dementia. Neurology. 2005 Apr 26; 64(8): 1378-83.

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Reitz C, Tang MX, Manly J, Schupf N, Mayeux R, Luchsinger JA. Plasma Lipid Levels and the Risk of Mild Cognitive Impairment. Submitted.

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1

Introduction

One of the earliest known written reports on dementia is attributed to Pythagoras in the 7th century BC, who described old age as a period of decline and decay of the human body and regression of mental capacities.[1] In 1907, Alois Alzheimer, a german psychiatrist and scientist, observed at necropsy an overload of - at that time still unknown - amyloid plaques and neurofibrillary tangles in the brain of a 51 year old woman who had suffered during her life course from progressive cognitive decline.[2] Nowadays, amyloid plaques and neurofibrillary tangles are considered the main neuropathological hallmarks of Alzheimer's disease, which is regarded the most frequent subtype of dementia.

Over the past two decades evidence has been accumulating that dementia is a heterogeneous and multifactorial disorder, and that besides accumulation of beta amyloid and neurofibrillary tangles, other factors, in particular vascular risk factors and cerebrovascular disease, may be involved, especially in late-onset dementia. Observational studies reported associations between several vascular risk factors and cognitive decline and dementia.[3] In autopsy studies, about 35% of the brains of elderly persons, who had been diagnosed with dementia during their lifetime, had not only a higher burden of amyloid plaques and neurofibrillar tangles but rather a mixed pathology also consisting of significant cerebrovascular disease.[4] Stroke has been reported to considerably increase the risk of dementia, with prevalence rates of post-stroke dementia of about 30%, reflecting a 3.6 to 5.8-fold increased risk of dementia compared to stroke-free subjects.[4] Cerebral small-vessel disease, which is defined as cerebral white matter lesions and asymptomatic lacunar brain infarcts and is a common finding on brain scans of elderly persons, has been reported to more than double the risk of dementia.[4-6]

Most of the evidence relating vascular risk factors and cerebrovascular disease with cognitive decline and dementia comes from cross-sectional studies, studies with a short follow-up time, autopsy studies, and stroke cohorts not taking accurately assessed pre-stroke cognitive function into account. However, dementia has a long preclinical period and diverse pathological changes contribute to the clinical symptoms of dementia. Further, it is uncertain whether factors that are more frequently observed in persons with dementia than non-demented persons, such as vascular risk factors or cerebrovascular disease, are a direct cause of dementia, rather precipitate dementia in an additive manner, or simply reflect coexisting disease. These facts demand studies with a longer follow-up to disentangle causes and consequences in the association between vascular disease and cognitive decline.

The objective of the work described in this thesis was to gain more insight into vascular and genetic risk factors underlying dementia etiology, and to further clarify the impact of cerebrovascular disease on risk of cognitive decline. I performed these studies in three different cohorts: a) the Rotterdam Study by the Department of Epidemiology & Biostatistics at Erasmus Medical Center Rotterdam; b) the Rotterdam Scan Study by the Department of Epidemiology & Biostatistics at Erasmus Medical Center Rotterdam; and c) the Washington

Heights Inwood Columbia Aging Project (WHICAP) by the G.H. Sergievsky Center at Columbia University, New York.

The Rotterdam Study is a population-based prospective study among 7,983 residents of Onmoord, a district of the city of Rotterdam, aged 55 years or older, that investigates the incidence and causes of cardiovascular, neurodegenerative, locomotor, and ophthalmologic diseases in the elderly.[7]

The Rotterdam Scan Study is a population-based prospective MRI study that included 1,077 non-demented persons aged 60 to 90 years and was designed to explore causes and consequences of brain changes on MRI in the elderly.[8] All participants underwent a brain MRI in 1995 to 1996, 668 participants underwent a second MRI more than three years later.

The WHICAP Study is a prospective cohort study among 4,316 randomly sampled Medicare recipients 65 years or older residing in northern Manhattan, that was designed to identify cognitive decline and its causes in elderly persons.[9] The participants were recruited at two time periods, 2,126 participants were recruited in 1992-1994 and 2,190 participants were recruited in 1999-2002.

To further clarify the impact of vascular disease on the risk of cognitive decline, I first explored the association between the endophenotypes of various vascular risk factors and the risk of different stages of cognitive impairment. This work is described in chapter 2 of this thesis. Then, as described in chapter 3, I assessed the impact of variation in genes encoding C-reactive protein and Matrix metalloproteinase 3 - factors involved in inflammation and vascular pathology - on the risk of cognitive impairment and cerebral small-vessel disease. As described above, cerebral small-vessel disease has been reported to be associated with an increased risk of cognitive decline and dementia.[5,6] Study of the association of genetic variation in genes encoding for vascular risk factors with vascular disease and cognitive decline provides the ability to further elucidate the role of these risk factors taking residual confounding into account. If a factor is causally involved in the development of a certain disease, the disease must not only be associated with the endophenotype of the factor but also with some genetic variation in its encoding gene.

Finally, I explored the direct impact of cerebrovascular disease on the risk of cognitive impairment and dementia. This work is described in chapter 4. First, I related stroke with the slope of cognitive test performance over time, then I related incident stroke with the risk of post-stroke dementia taking pre-stroke cognitive performance into account.

In chapter 5, I review the main results of the studies described in this thesis and discuss them in the context of current knowledge and potential methodological limitations. Finally, I give suggestions for future research.

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2

Vascular risk factors and cognitive decline

2.1

Relation of Plasma Lipids to Alzheimer's Disease and Vascular Dementia

ABSTRACT

Context. The relation between plasma lipids, Alzheimer's disease and vascular dementia, and the impact of lipid lowering drugs remains unclear. **Objective.** To investigate the relation between plasma lipid levels and the risk of Alzheimer's disease and vascular dementia, and the impact of lipid lowering drugs on this relationship. **Design and Setting.** Cross-sectional and prospective community-based cohort studies. **Participants.** Random sample of 4316 Medicare recipients, 65 years and older, residing in northern Manhattan. **Main Outcome Measures.** Vascular dementia and Alzheimer's disease according to standard criteria. **Results.** Elevated levels of non-HDL cholesterol and LDL as well as decreased levels of HDL were weak risk factors for vascular dementia in either cross-sectional or prospective analysis. Higher levels of total cholesterol were associated with a decreased risk of incident Alzheimer's disease after adjustment for demographics, APOE genotype and cardiovascular risk factors. Treatment with lipid lowering drugs did not change the disease risk of either disorder. **Conclusion.** We found a weak relation between non-HDL cholesterol, LDL and HDL levels and the risk of vascular dementia. Lipid levels and the use of lipid lowering agents do not seem to be associated with the risk of Alzheimer's disease.

INTRODUCTION

The prevalence of dementia is increasing in western societies and there are no known measures to prevent or cure it. There is conflicting data showing that dyslipidemia, a modifiable risk factor, is associated with a higher risk of dementia. Reduced high-density lipoprotein cholesterol (HDL-C)[1,2] and apolipoprotein A-1 levels,[3] as well as increased levels of lipoprotein (a)[3] have been observed in vascular dementia (VaD) in some but not all studies.[4,5] There also have been contradictory results in studies relating total cholesterol,[6,7] HDL[3,8,9] and low-density lipoprotein (LDL) cholesterol[6,8] with Alzheimer's disease (AD).

Interest in these relationships has been increased by the observation that widely available lipid lowering agents, particularly HMG-COA-reductase-inhibitors (statins), may lower the risk of AD[10] or VaD,[11] and that cholesterol alters the degradation of the amyloid precursor protein (APP), which plays a major role in the pathogenesis of AD.[12] Moreover, cerebrovascular disease, which is associated with dyslipidemia may be related to the risk of AD.[13] We previously reported an association between high levels of total and LDL[14] and VaD, but no association of LDL with AD. Our objective in this study was to explore these associations in a larger cross-sectional study and a prospective study with longer follow-up, and assess the association between lipid lowering agents and dementia.

METHODS

Participants and Setting. Participants were enrolled in a longitudinal cohort study by a random sampling of medicare recipients 65 years or older residing in northern Manhattan

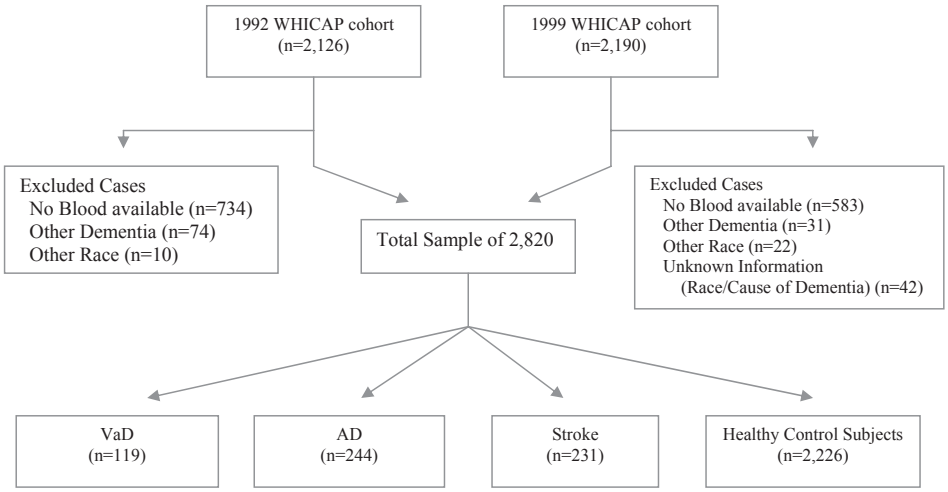

Figure 1a. Description of cross-sectional sample

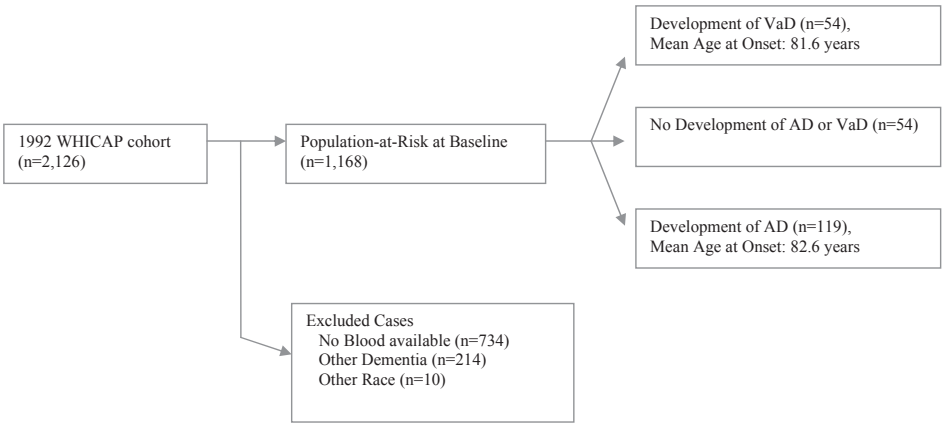

Figure 1b. Description of sample used in prospective study

(Washington Heights, Hamilton Heights, Inwood). The sampling procedures have been described elsewhere.[15] Each participant underwent an in-person interview of general health and function at the time of study entry followed by a standard assessment, including medical history, physical and neurological examination as well as a neuropsychological battery.[16] Ethnic origin was classified by self-report using the format of the 1990 US-Census.[17] Participants were recruited at two time periods (1992-1994 and 1999-2002). They have been followed at approximately 18 month intervals with similar assessments at each follow-up. The institutional review board of Columbia-Presbyterian Medical Center approved this study.

Of the 4316 individuals who underwent clinical assessment at baseline, we excluded in the cross-sectional analysis data from 1496 individuals (34.6%) (figure 1a). Plasma lipids were unavailable in 1317 cases, because lipid levels were obtained during the second follow-up visit. Information on ethnic group and cause of dementia was unknown in 42 cases. 105 had other causes of dementia (not AD or VaD) and 32 were members of an ethnic group other than African-American, White (European American) or Carribean Hispanic. The final analytic sample in the cross-sectional analysis contained a total of 2820 participants.

The prospective study included only participants from the 1992 cohort. Of the 2126 subjects who underwent clinical assessment at baseline, we excluded data from 958 individuals (45.1%) (figure 1b). Plasma lipids were unavailable in 734 cases, because lipid levels were obtained during the second follow-up visit. 10 individuals were members of an ethnic group other than African-American, White (European American) or Carribean Hispanic. 214 subjects were excluded due to prevalent dementia. The final analytic sample contained a total of 1168 participants.

Diagnosis of Stroke. Stroke was defined according to the WHO criteria.[18] The diagnosis was based on questioning of the participant and/or relatives, supplemented by a neurological

examination and/or review of medical records. Results of brain imaging were available on 85% of those with VaD.

Diagnosis of Dementia. The diagnosis of dementia was established based on all available information gathered from the initial and follow-up assessments and medical records. Dementia was determined by consensus at a conference of physicians, neurologists, neuropsychologists and psychiatrists. The diagnosis of dementia was based on standard research criteria and required evidence of cognitive decline, including memory impairment, on the neuropsychological test battery as well as evidence of impairment in social or occupational function (clinical dementia rating > 0.5).[19]

A diagnosis of VaD was considered for individuals with dementia combined with a history or clinical evidence of stroke and was classified as follows:[20] (1) stroke related dementia (eg, new onset of dementia within 3 months of a stroke), (2) dementia due to focal effects of a stroke (eg, dementia resulting from stroke(s) in strategic area(s) whose singular or additive effects accounted for the cognitive impairment), and (3) possible AD with concomitant stroke (eg, progressive dementia associated with a clinical history of stroke in which the temporal relationship could not be established).

The diagnosis of AD was based on the National Institute of Neurological and Cognitive Disorders and Stroke/Alzheimer's Disease and Related Disorders Association Criteria.[21]

Diabetes mellitus, Heart Disease and Hypertension. Diabetes and Hypertension were defined as a history of either disorder at any time during life. At baseline, all participants were asked whether or not they had a history of diabetes or hypertension. If affirmed, they were asked whether or not they were under treatment and the specific type of medication. Heart disease was defined as a history of myocardial infarction, congestive heart failure or angina pectoris at any time during life.

Treatment with lipid lowering drugs (statins). At baseline, all participants were asked if they ever have been treated with lipid lowering drugs. If affirmed, they were asked for the specific type of drug.

Plasma Lipids and APOE Genotyping. Fasting plasma total cholesterol and triglyceride levels were determined at initial assessment using standard enzymatic techniques. HDL cholesterol levels were determined after precipitation of apolipoprotein B containing lipoproteins with phosphotungstic acid.[22] LDL was recalculated using the formula of Friedewald et al.[23] APOE genotypes were determined as described by Hixson and Vernier[24] with slight modification.[25] We classified persons as homozygeous or heterozygeous for the APOE ε4 allele or not having any ε4 allele.

Statistical Methods. Lipid levels and other potentially relevant factors were compared among individuals with VaD, AD, stroke and healthy control subjects in the cross-sectional and prospective samples. χ^2 tests were used for categorical data and analysis of variance for continuous variables. Because the distribution of HDL and triglycerides was skewed, logarithmic transformation of these data was carried out and statistical tests were repeated.

In the cross-sectional analysis we included participants of both the 1992 and 1999 cohorts. Logistic regression was used to estimate the odds ratio (OR) of dementia (AD or VaD) associated with plasma lipid levels. Plasma lipids were analysed first as continuous variables and later grouped into quartiles. After adjusting for gender, age, ethnicity and education, we performed a second model adjusting for body mass index, diabetes mellitus, hypertension, heart disease and APOEε4 genotype. To estimate the effect of lipid lowering treatment, separate analyses were performed for treated respectively not treated individuals.

The prospective study included only participants from the 1992 cohort. Proportional hazard models were used to estimate the association of plasma lipid levels with the incidence of AD and VaD. The time-to-event variable was age-at-onset of dementia. Data from individuals who did not develop AD or VaD or who died or were lost to follow-up prior to developing dementia were censored at the time of their last evaluation.

Information on covariates was obtained at baseline. After adjusting for gender, age, race and education we adjusted for body mass index, diabetes mellitus, hypertension, heart disease and APOEε4 genotype in a second model. Separate models were performed for treated and not treated individuals. Data analysis was performed using SPSS version 11.0.

RESULTS

Cross-sectional analysis

First we performed a cross-sectional analysis of the 1992 and 1999 cohorts. Lipid levels, demographics and vascular risk factors were compared among individuals with VaD, AD, stroke and healthy control subjects.

The mean age of the sample was 77.2 ± 6.7 years. 66.7% were women, 25.7% were white, 32.0% black and 42.3% were hispanic. The median of years of education was 9. The mean level of total cholesterol was 198.8, of non-HDL cholesterol 151.4, of HDL 47.4, of triglycerides 155.9 and of LDL 120.1 mg/dl. 28.7% of the cohort were heterozygeous or homozygeous for the APOEε4 allele. 19.9% had a history of diabetes, 23.3% a history of heart disease and 62.0% a history of hypertension. Use of lipid lowering agents was reported by 477 subjects (16.9%).

Women had higher levels of total cholesterol, non-HDL cholesterol, HDL and LDL than men (table 1). Hispanics had significantly lower levels of total cholesterol than Whites. They had

Table 1. Comparison of lipid levels by demographics in 2,820 subjects

	Cholesterol (mg/dl)	Non-HDL cholesterol (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)
Men	188.34 (38.5)	145.61 (37.7)	42.45 (14.1)	156.28 (89.7)	114.69 (33.4)
Women	204.15 (39.9) *	154.22 (39.6)*	49.97 (14.8) *	155.71 (83.7)	122.92 (35.3) *
Ethnic group †					
White/Non-Hispanic	201.59 (39.2) *	154.32 (37.9)*	47.41 (14.5) *	155.74 (79.4) *	122.90 (33.0) *
Black/Non-Hispanic	199.56 (38.1)	147.62 (36.4)	51.94 (15.7) **	128.30 (65.9)	121.95 (34.0) *
Hispanic	196.74 (42.2)	152.61 (39.1)	44.12 (13.8)	176.89 (96.3)**	117.19 (36.5)

Values are expressed as number (SD) unless otherwise indicated. Some percentages are based on an incomplete sample due to small amounts of missing data. * Significant at a 0.05 level versus lowest value within lipid group, based on analysis of variance for continuous data and χ^2 test for categorical data. ** Significant at a 0.05 level versus all lower values within lipid group, based on analysis of variance for continuous data and χ^2 test for categorical data. † Classified by self-report using the format of the 1990 US census.[17]

lower levels of HDL and LDL and higher levels of triglycerides than Whites and Blacks. Blacks had higher levels of HDL and lower levels of non-HDL cholesterol and triglycerides than Whites.

The subjects with VaD or AD were significantly older and less educated than individuals with stroke without dementia or control subjects (table 2). The VaD group had significantly more Hispanics than Whites and the AD group had more Hispanics and Blacks than Whites. A history of diabetes, heart disease and hypertension was more frequent in the stroke and VaD group compared with the control group. The APOEε4 genotype was significantly more frequent in the AD group compared with the control group.

Plasma Lipid Levels and the Risk of AD. There was no association between plasma lipids and a higher risk of AD (table 3). Adjustment for demographics, cardiovascular risk factors and APOE genotype did not change this relation. Treatment with lipid lowering drugs was negatively associated with the risk of AD (OR 0.45, 95% CI 0.27-0.75, p=0.002).

Plasma Lipid Levels and the Risk of VaD. Both HDL and non-HDL cholesterol were associated with the risk of VaD. The prevalence of VaD decreased with higher levels of HDL (OR 0.47; 95% CI 0.26-0.83, p for trend = 0.01) while it increased with higher levels of non-HDL cholesterol (OR 1.21, 95% CI 1.011-1.447, p for trend= 0.037) (table 4). The strength of these associations was similar in men and women. Treatment with lipid lowering agents was not associated with the risk of prevalent VaD (OR 0.87; 95% CI 0.49-1.56, p=0.65).

Table 2. Comparison of characteristics among outcome group in 2,820 subjects in the cross-sectional analysis

	Vascular dementia (n=119)	Alzheimer's disease (n=244)	Stroke without dementia (n=231) †	Control subjects (n=2226)
Men	36 (30.3)	55 (22.5)	87 (37.7)	760 (34.1)
Women	83 (69.7)	189 (77.5) *	144 (62.3)	1466 (65.9)
Education, mean (SD), year	6.61 (4.1)*	6.33 (4.3)*	9.85 (4.8)	9.74 (4.7)
Age, mean (SD), year	80.42 (6.9)*	82.85 (7.3)*	77.66 (6.2)	76.42 (6.3)
Body mass index, mean (SD)	26.34 (5.3)	26.42 (5.7)	27.25 (5.9)	27.59 (6.8)
Ethnic group ‡				
White/Non-Hispanic	8 (6.7)	19 (7.8)	57 (24.7)	642 (28.8)
Black/Non-Hispanic	39 (32.8)	96 (39.3) *	78 (33.8)	689 (31.0)
Hispanic	72 (60.5) *	129 (52.9) *	96 (41.6)	895 (40.2)
APOE genotype 4/4	3 (3.4)	9 (4.4) *	1 (0.7)	26 (1.8)
APOE genotype 4/-	29 (33.3)	72 (35.5) *	48 (35.0)	355 (24.2)
APOE genotype -/-	55 (63.2)	122 (60.1)	88 (64.2)	1085 (74.0)
Cholesterol (mg/dl), mean (SD)	199.76 (44.7)	197.11 (41.2)	199.48 (45.3)	198.98 (39.3)
Non-HDL cholesterol (mg/dl), mean (SD)	155.3 (44.9)	149.6 (39.1)	152.8 (44.7)	151.3 (38.2)
HDL (mg/dl), mean (SD)	44.42 (14.2)	47.49 (15.6)	46.97 (17.0)	47.68 (14.7)
Triglycerides (mg/dl), mean (SD)	165.03 (83.7)	147.51 (84.1)	162.27 (100.3)	155.67 (84.4)
LDL (mg/dl), mean (SD)	122.33 (37.9)	120.11 (35.8)	119.36 (37.6)	120.16 (34.4)
No Diabetes	85 (71.4) *	188 (77.0)	170 (74.6) *	1727 (82.0)
Diabetes, not treated	9 (7.6) *	13 (5.3)	15 (6.6)	71 (3.4)
Diabetes, treated	25 (21.0) *	43 (17.6)	43 (18.9) *	308 (14.6)
No heart disease	82 (68.9) *	198 (81.1)	142 (62.0) *	1649 (78.3)
Heart disease, not treated	13 (10.9) *	22 (9.0)	34 (14.8) *	141 (6.7)
Heart disease, treated	24 (20.2) *	24 (9.8)	53 (23.1) *	317 (15.1)
No hypertension	35 (29.4) *	99 (40.7)	51 (22.3) *	836 (39.9)
Hypertension, not treated	25 (21.0) *	38 (15.6)	35 (15.3)	231 (11.0)
Hypertension, treated	59 (49.6)	106 (43.6)	143 (62.4) *	1029 (49.1)

Values are expressed as number (percentage) unless otherwise indicated. Some percentages are based on an incomplete sample due to small amounts of missing data. * Significant at a 0.05 level versus control group, based on analysis of variance for continuous data and χ^2 test for categorical data. † defined according to World Health Organisation Criteria.[18] ‡ Classified by self-report using the format of the 1990 US census.[17]

Table 3. Odds ratios and 95% confidence intervals, relating plasma lipids and the risk of prevalent AD

Quartiles range (mg/dl)	AD (n=244) (%)	Control subjects (n=2226) (%)	B*	S.E. *	Sig. *	OR (95% CI) *	OR (95% CI) †
Cholesterol							
1 (≤ 172.00)	72 (29.5)	552(24.8)				1.0	
2 (172.01-197.00)	63 (25.8)	565 (25.4)	0.062	0.202	0.758	1.064 (0.716-1.582)	0.964 (0.607-1.529)
3 (197.01-225.00)	56 (23.0)	552 (24.8)	-0.193	0.210	0.357	0.824 (0.546-1.244)	0.715 (0.440-1.160)
4 (≥ 225.01)	53 (21.7)	557 (25.0)	-0.117	0.211	0.579	0.889 (0.588-1.346)	0.939 (0.581-1.517)
trend test						p = 0.368	p = 0.522
Non-HDL cholesterol							
1 (≤ 124.00)	71 (29.1)	562 (25.2)				1.0	1.0
2 (124.01-149.00)	65 (26.6)	555 (24.9)	0.122	0.197	0.536	1.130 (0.767-1.663)	1.148 (0.730-1.808)
3 (149.01-176.00)	54 (22.1)	575 (25.8)	-0.117	0.205	0.569	0.890 (0.596-1.329)	0.876 (0.546-1.407)
4 (≥ 176.01)	54 (22.1)	534 (23.9)	0.006	0.206	0.977	1.006 (0.672-1.507)	1.050 (0.654-1.684)
trend test						p=0.736	p=0.863
HDL							
1 (≤ 37.00)	69 (29.2)	494 (23.4)				1.0	1.0
2 (37.01-45.00)	46 (19.5)	555 (26.3)	-0.655	0.224	0.003	0.519 (0.335-0.805)	0.470 (0.282-0.785)
3 (45.01-55.00)	51 (21.6)	513 (24.3)	-0.501	0.225	0.026	0.606 (0.390-0.942)	0.582 (0.350-0.969)
4 (≥ 55.01)	70 (29.7)	552 (26.1)	-0.296	0.213	0.164	0.744 (0.490-1.129)	0.664 (0.407-1.085)
trend test						p = 0.344	p = 0.219
Triglycerides							
1 (≤ 97.00)	73 (30.3)	548 (24.8)				1.0	
2 (97.01-135.00)	65 (27.0)	536 (24.3)	-0.162	0.205	0.430	0.851 (0.570-1.271)	0.931 (0.576-1.504)
3 (135.01-191.00)	50 (20.7)	578 (26.2)	-0.425	0.217	0.050	0.654 (0.427-1.001)	0.798 (0.484-1.315)
4 (≥ 191.01)	53 (22.0)	545 (24.7)	-0.194	0.218	0.374	0.824 (0.537-1.263)	0.951 (0.578-1.565)
trend test						p = 0.209	p = 0.718
LDL							
1 (≤ 96.50)	69 (28.3)	550 (24.7)				1.0	
2 (96.51-118.80)	59 (24.2)	559 (25.1)	0.100	0.207	0.630	1.105 (0.736-1.657)	0.872 (0.542-1.402)
3 (118.81-142.80)	57 (23.4)	565 (25.4)	-0.096	0.209	0.647	0.909 (0.603-1.368)	0.845 (0.525-1.361)
4 (≥ 142.81)	59 (24.2)	552 (24.8)	-0.017	0.208	0.936	1.017 (0.676-1.529)	1.022 (0.633-1.650)
trend test						p = 0.834	p = 0.985

Logistic regression. Values are expressed as number (percentage) unless otherwise indicated. Some percentages are based on an incomplete sample due to small amounts of missing data. B=estimated logistic regression coefficient, S.E.=standard error, Sig.=significance, OR=odds ratio, 95% CI= 95 percent confidence interval. * adjusting for gender, age, education and race . † adjusting for body mass index, APOE, diabetes, heart disease and hypertension.

Table 4. Odds ratios and 95% confidence intervals, relating plasma lipids and the risk of prevalent VaD

Quartiles range (mg/dl)	VaD (n=119) (%)	Control subjects (n=2226) (%)	B*	S.E. *	Sig. *	OR (95% CI)*	OR (95% CI) †
Cholesterol							
1 (≤ 172.00)	27 (22.7)	552(24.8)				1.0	
2 (172.01-197.00)	33 (27.7)	565 (25.4)	0.421	0.277	0.129	1.523 (0.885-2.621)	1.394 (0.699-2.780)
3 (197.01-225.00)	23 (19.3)	552 (24.8)	0.065	0.302	0.829	1.067 (0.591-1.927)	0.798 (0.360-1.769)
4 (≥ 225.01)	36 (30.3)	557 (25.0)	0.570	0.276	0.039	1.768 (1.030-3.035)	1.677 (0.829-3.393
trend test						p = 0.107	p = 0.336
Non-HDL cholesterol							
1 (≤ 124.00)	30 (25.2)	562 (25.2)				1.0	1.0
2 (124.01-149.00)	21 (17.6)	555 (24.9)	-0.128	0.317	0.687	0.880 (0.473-1.637)	0.959 (0.447-2.057)
3 (149.01-176.00)	34 (28.6)	575 (25.8)	0.373	0.282	0.187	1.452 (0.835-2.524)	1.098 (0.517-2.331)
4 (≥ 176.01)	34 (28.6)	534 (23.9)	0.471	0.284	0.097	1.602 (0.919-2.793)	1.549 (0.753-3.187)
trend test						p=0.037	p=0.223
HDL							
1 (≤ 37.00)	42 (36.5)	494 (23.4)				1.0	
2 (37.01-45.00)	24 (20.9)	555 (26.3)	-0.778	0.276	0.005	0.459 (0.267-0.789)	0.619 (0.306-1.249)
3 (45.01-55.00)	25 (21.7)	513 (24.3)	-0.629	0.281	0.025	0.533 (0.307-0.925)	0.775 (0.382-1.573)
4 (≥ 55.01)	24 (20.9)	552 (26.1)	-0.752	0.289	0.009	0.472 (0.268-0.830)	0.599 (0.282-1.275)
trend test						p = 0.016	p = 0.274
Triglycerides							
1 (≤ 97.00)	22 (18.5)	548 (24.8)				1.0	
2 (97.01-135.00)	39 (32.8)	536 (24.3)	0.566	0.288	0.049	1.761 (1.002-3.097)	1.253 (0.611-2.569)
3 (135.01-191.00)	21 (17.6)	578 (26.2)	-0.147	0.326	0.652	0.863 (0.456-1.636)	0.784 (0.351-1.751)
4 (≥ 191.01)	37 (31.1)	545 (24.7)	0.624	0.299	0.037	1.866 (1.083-3.355)	1.393 (0.663-2.927)
trend test						p = 0.230	p = 0.618
LDL							
1 (≤ 96.50)	26 (21.8)	550 (24.7)				1.0	
2 (96.51-118.80)	29 (24.4)	559 (25.1)	0.285	0.286	0.320	1.330 (0.758-2.331)	0.927 (0.445-1.930)
3 (118.81-142.80)	30 (25.2)	565 (25.4)	0.287	0.284	0.312	1.332 (0.764-2.324)	1.200 (0.590-2.439)
4 (≥ 142.81)	34 (28.6)	552 (24.8)	0.532	0.279	0.057	1.702 (0.985-2.940)	1.399 (0.679-2.880)
trend test						p = 0.069	p = 0.275

Logistic regression. Values are expressed as number (percentage) unless otherwise indicated. Some percentages are based on an incomplete sample due to small amounts of missing data. B=estimated logistic regression coefficient, S.E.=standard error, Sig.=significance, OR=odds ratio, 95% CI= 95 percent confidence interval. * adjusting for gender, age, education and race. † adjusting for body mass index, APOE, diabetes, heart disease and hypertension.

Prospective analysis

Subsequently we performed a proportional hazard model of the 1992 cohort. The mean age of the sample was 78.4 ± 6.2 years. 68.3% were women, 20.4% were white, 31.8% black and 47.9% hispanic. The median of years of education was 8. The mean level of total cholesterol was 203.1, of non-HDL cholesterol 156.1, of HDL 47.0, of triglycerides 185.4 and of LDL 118.9 mg/dl. 27.3% of the cohort were heterozygeous or homozygeous for the APOEε4 allele. 17.9% had a history of diabetes, 16.1% a history of heart disease and 55.1% a history of hypertension. Use of lipid lowering agents was reported by 136 subjects (11.6%). There were 54 cases of incident VaD and 119 cases of incident AD during 5189 person years of observation. The mean duration of observation was 4.8 ± 2.9 years.

Individuals who developed either AD or VaD at follow up were significantly less educated, older and more often Hispanic or Black than White compared with controls (table 5). Individuals who developed AD had a higher frequency of an APOEε 4 genotype and at baseline significantly lower levels of total cholesterol than controls. Individuals who developed VaD were more often women than men, had higher non-HDL cholesterol levels than individuals who developed AD or remained free of dementia and were more likely to have a history of diabetes and heart disease compared with controls.

Risk of incident vascular dementia. The mean age at onset of VaD was 81.6 years. Both LDL and non-HDL cholesterol were associated with VaD (table 6). The risk of VaD increased with increasing quartile of non-HDL cholesterol (HR 1.327, 95% CI 1.009-1.743, p for trend=0.043) and LDL (HR 2.48; 95 % CI 1.05-5.70; p for trend = 0.04). The strength of these associations was similar in men and women. Treatment with lipid lowering agents was not associated with the risk of incident VaD (HR 1.45; 95% CI 0.65-3.28, p=0.36).

Risk of Alzheimer's disease. The mean age of onset of AD was 82.6 years. Higher levels of total cholesterol were associated with a lower risk of incident AD after adjustment for demographics and body mass index, APOE genotype, diabetes, heart disease and hypertension (HR 0.48; 95 % CI 0.26-0.86; p for trend = 0.04) (table 7). No other plasma lipid was associated with AD risk. Treatment with lipid lowering agents was not associated with the risk of incident AD (HR 0.88; 95% CI 0.44-1.76, p=0.725).

COMMENT

In our cross-sectional analysis of 2820 subjects we found that higher non-HDL levels and lower HDL levels were associated with a higher risk of VaD but not AD. We also found an association between higher LDL levels and a higher risk of VaD that was close to statistical

Table 5. Comparison of characteristics among outcome group in 1,168 subjects followed prospectively

	Incident vascular dementia (n=54)	Incident Alzheimer's disease (n=119)	Control subjects (n=856)
Men	11 (20.4) *	40 (36.6)	276 (32.2)
Women	43 (79.6) *	79 (66.4)	580 (67.8)
Education, mean (SD), year	7.30 (4.0) *	6.75 (4.6) *	8.83 (4.6)
Age, mean (SD), year	80.05 (6.6) *	81.49 (7.2) *	77.81 (5.9)
Body mass index, mean (SD)	27.29 (6.8)	27.09 (5.7)	27.57 (5.5)
Ethnic group †			
White/Non-Hispanic	3 (5.6) *	10 (8.4) *	199 (32.2)
Black/Non-Hispanic	21 (38.9) *	46 (38.7) *	264 (30.8)
Hispanic	30 (55.6) *	63 (52.9) *	393 (45.9)
APOE genotype 4/4	1 (2.0)	8 (6.9) *	13 (1.6)
APOE genotype 4/-	17 (33.3)	30 (25.9)	203 (24.7)
APOE genotype -/-	33 (64.7)	78 (67.2) *	607 (73.8)
Cholesterol (mg/dl), mean (SD)	210.44 (35.5)	194.02 (41.0) *	204.02 (39.9)
Non-HDL cholesterol (mg/dl), mean (SD)	165.11 (39.3)**	147.95 (38.9)	156.53 (40.6)
HDL (mg/dl), mean (SD)	46.75 (12.2)	46.59 (14.5)	47.45 (15.9)
Triglycerides (mg/dl), mean (SD)	187.72 (86.8)	169.62 (77.5)	186.68 (96.2)
LDL (mg/dl), mean (SD)	126.42 (32.3)	112.95 (35.9)	119.14 (36.3)
No Diabetes	37 (68.5) *	96 (80.7)	635 (84.7)
Diabetes, not treated	4 (7.4) *	5 (4.2)	29 (3.9)
Diabetes, treated	13 (24.1) *	18 (15.1)	86 (11.5)
No heart disease	41 (75.9) *	98 (82.4)	652 (86.9)
Heart disease, not treated	5 (9.3) *	5 (4.2)	28 (3.7)
Heart disease, treated	8 (14.8) *	16 (13.4)	70 (9.3)
No hypertension	23 (42.6)	58 (48.7)	345 (46.3)
Hypertension, not treated	13 (24.1)	22 (18.5)	125 (16.8)
Hypertension, treated	18 (33.3)	39 (32.8)	275 (36.9)

Values are expressed as number (percentage) unless otherwise indicated. Some percentages are based on an incomplete sample due to small amounts of missing data. * Significant at a 0.05 level versus control group, based on analysis of variance for continuous data and χ^2 test for categorical data. ** Significant at a 0.05 level versus Alzheimer's Disease group, based on analysis of variance for continuous data and χ^2 test for categorical data. † Classified by self-report using the format of the 1990 US census.[17]

significance, but higher LDL levels were not related to AD risk. Treatment with lipid lowering agents was negatively associated with the risk of AD but not VaD. In a longitudinal analysis of 1168 subjects (5189 person-years of follow-up) we observed an association between higher LDL levels and a higher risk of VaD but not AD and replicated the association between higher non-HDL levels and a higher risk of VaD found in the cross-sectional analysis. Moreover we found an association between higher cholesterol levels and a lower risk of AD. We did not

Table 6. Hazard ratios and 95% confidence intervals, relating plasma lipids and the risk of incident VaD

Quartiles range (mg/dl)	At-Risk population (%)	No. (%) of Incident VaD	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Cholesterol				
1 (≤ 176.00)	293 (25.1)	9 (3.1)	1.0	1.0
2 (176.01-202.50)	291 (24.9)	12 (4.1)	1.051 (0.440-2.509)	0.783 (0.317-1.938)
3 (202.51-229.00)	293 (25.1)	16 (5.4)	1.684 (0.715-3.965)	1.572 (0.651-3.794)
4 (≥ 229.01)	291 (24.9)	17 (5.8)	1.614 (0.697-3.738)	1.049 (0.423-2.602)
trend test			p= 0.162	p= 0.558
Non-HDL cholesterol				
1 (≤ 128.00)	299 (25.6)	11 (3.7)	1.0	1.0
2 (128.01-154.00)	285 (24.4)	12 (4.2)	1.192 (0.482-2.947)	1.082 (0.418-2.804)
3 (154.01-182.00)	296 (25.4)	9 (3.0)	0.985 (0.373-2.602)	0.935 (0.340-2.570)
4 (≥ 182.01)	285 (24.4)	21 (7.4)	2.375 (1.050-5.373)	2.007 (0.844-4.773)
trend test			p=0.043	p=0.130
HDL				
1 (≤ 36.00)	266 (24.1)	11 (4.1)	1.0	1.0
2 (36.01-45.00)	309 (27.9)	15 (4.8)	1.035 (0.467-2.293)	0.922 (0.390-2.178)
3 (45.01-55.00)	260 (23.5)	10 (3.8)	0.525 (0.209-1.318)	0.575 (0.212-1.554)
4 (≥ 55.01)	271 (24.5)	16 (5.9)	0.840 (0.363-1.946)	0.808 (0.319-2.049)
trend test			p= 0.445	p= 0.543
Triglycerides				
1 (≤ 121.00)	295 (25.5)	12 (4.1)	1.0	1.0
2 (121.01-161.00)	283 (24.5)	13 (4.6)	0.986 (0.439-2.213)	0.904 (0.377-2.172)
3 (161.01-224.00)	288 (24.9)	12 (4.2)	1.226 (0.540-2.781)	1.002 (0.395-2.544)
4 (≥ 224.01)	290 (25.1)	16 (5.5)	1.489 (0.668-3.318)	1.337 (0.546-3.273)
trend test			p= 0.275	p= 0.466
LDL				
1 (≤ 94.35)	290 (24.9)	8 (2.8)	1.0	1.0
2 (94.36-117.30)	291(25.0)	12 (4.1)	1.634 (0.662-4.037)	1.569 (0.629-3.911)
3 (117.31-142.75)	291 (25.0)	14 (4.8)	1.610 (0.660-3.926)	1.116 (0.430-2.896)
4 (≥ 142.76)	291 (25.0)	19 (6.5)	2.447 (1.051-5.701)	2.074 (0.851-5.056)
trend test			p= 0.043	p= 0.175

Cox proportional hazards model, with age-at-onset as time variable, as described in the text. Some percentages are based on an incomplete sample due to small amounts of missing data. HR=hazard ratio, 95% CI= 95 percent confidence interval. Model 1: adjusting for gender, age, education and race. Model 2: adjusting for body mass index, APOE, diabetes, heart disease and hypertension

replicate the association between HDL and risk of VaD found in the cross-sectional analysis in the prospective analysis. We also did not replicate the negative association of lipid lowering agents with the risk of AD found in the cross-sectional analysis.

Table 7. Hazard ratios and 95% confidence intervals, relating plasma lipids and the risk of incident AD

Quartiles range (mg/dl)	At-Risk population (%)	No. (%) of Incident AD	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Cholesterol				
1 (≤ 176.00)	293 (25.1)	43 (14.6)	1.0	1.0
2 (176.01-202.50)	291 (24.9)	26 (8.9)	0.632 (0.385-1.038)	0.578 (0.343-0.974)
3 (202.51-229.00)	293 (25.1)	29 (9.8)	0.853 (0.518-1.405)	0.823 (0.481-1.406)
4 (≥ 229.01)	291 (24.9)	21 (7.2)	0.548 (0.315-0.952)	0.475 (0.264-0.855)
trend test			p= 0.072	p= 0.038
Non-HDL cholesterol				
1 (≤ 128.00)	299 (25.6)	41 (13.7)	1.0	1.0
2 (128.01-154.00)	285 (24.4)	28 (9.8)	0.761 (0.475-1.222)	0.792 (0.485-1.294)
3 (154.01-182.00)	296 (25.4)	31 (10.5)	0.845 (0.533-1.369)	0.877 (0.539-1.429)
4 (≥ 182.01)	285 (24.4)	19 (6.6)	0.608 (0.356-1.038)	0.599 (0.345-1.039)
trend test			p=0.105	p=0.109
HDL				
1 (≤ 36.00)	266 (24.1)	29 (10.9)	1.0	1.0
2 (36.01-45.00)	309 (27.9)	27 (8.7)	0.873 (0.506-1.506)	0.791 (0.442-1.417)
3 (45.01-55.00)	260 (23.5)	31 (11.9)	0.848 (0.493-1.457)	0.970 (0.538-1.749)
4 (≥ 55.01)	271 (24.5)	27 (9.9)	0.718 (0.407-1.265)	0.702 (0.374-1.320)
trend test			p= 0.265	p= 0.391
Triglycerides				
1 (≤ 121.00)	295 (25.5)	37 (12.5)	1.0	1.0
2 (121.01-161.00)	283 (24.5)	29 (10.2)	0.820 (0.497-1.353)	0.749 (0.442-1.271)
3 (161.01-224.00)	288 (24.9)	29 (10.1)	0.975 (0.583-1.630)	0.891 (0.509-1.559)
4 (≥ 224.01)	290 (25.1)	23 (7.9)	0.764 (0.438-1.335)	0.761 (0.418-1.386)
trend test			p= 0.481	p= 0.503
LDL				
1 (≤ 94.35)	290 (24.9)	36 (12.4)	1.0	1.0
2 (94.36-117.30)	291(25.0)	32 (10.9)	1.005 (0.619-1.629)	0.986 (0.590-1.647)
3 (117.31-142.75)	291 (25.0)	26 (8.9)	0.776 (0.461-1.307)	0.782 (0.458-1.336)
4 (≥ 142.76)	291 (25.0)	24 (8.2)	0.878 (0.510-1.510)	0.800 (0.458-1.396)
trend test			p= 0.426	p= 0.306

Cox proportional hazards model, with age-at-onset as time variable, as described in the text. Some percentages are based on an incomplete sample due to small amounts of missing data. HR=hazard ratio, 95% CI= 95 percent confidence interval. Model 1: adjusting for gender, age, education and race. Model 2: adjusting for body mass index, APOE, diabetes, heart disease and hypertension

The causal role of vascular risk factors in different types of dementia has been stressed during the last decade.[26] The sclerosis of small cerebral arteries and arterioles is considered to be responsible for diffuse periventricular white matter abnormalities, which play an important role in the development of VaD.[26] Dyslipidemia, a well-established risk factor for ischemic heart disease, has not yet been convincingly demonstrated as a factor associated with brain ischemia, VaD or AD. For example, several authors observed normal or low levels of total cholesterol or LDL in ischemic-stroke patients.[26]

There are different pathways in which plasma lipids could be associated with the risk of vascular dementia. High concentrations of LDL and low levels of HDL cholesterol are known to be independent risk factors for coronary heart disease[14] and carotid artery atherosclerosis,[27] which in turn may lead to cognitive impairment through cerebral hypoperfusion or embolism.[28] HDL particles might also be linked with small-vessel disease by playing a role in the removal of excess cholesterol from the brain by interaction with APOE and heparan sulfate proteoglycans in the subendothelian space of cerebral microvessels.[29] Second, the brain appears to be particularly vulnerable to oxidative lipid damage because of its high content of polyunsaturated fatty acids.[30,31,32] There is much evidence that decreased levels of antioxidants such as α-tocopherol, beta carotene, vitamin C or serum paroxonase lead to higher susceptibility to oxidative stress and a higher grade of LDL oxidation, and different studies have found evidence for lower levels of antioxidants in patients with VaD.[33,34] Moreover there is evidence that LDL peroxidation increases with age.[35]

The role of dyslipidemia in the development of AD remains unclear. Brain cholesterol alters the degradation of APP(2), which contributes to the pathogenesis of AD.[12] However, brain cholesterol is almost entirely synthesized in situ and not transferred from the plasma into the brain, due to the blood-brain-barrier.[36] There is also evidence that plasma cholesterol levels have no effect on brain HMG-CoA-reductase levels and its activity[9] or levels of 24S-hydroxycholesterol, which is a degradation product of brain cholesterol.[37] Moreover, reduced and not increased cellular cholesterol levels promote tau phosphorylation in neurons, inhibit dendrite outgrowth and synaptogenesis, and induce neurodegeneration.[9]

There are different pathways in which statins could lower the risk of dementia. Besides having a lowering effect on plasma lipid levels they could also lower the risk of dementia by their pleiotropic effects.[38] They can improve the endothelial function of atherosclerotic vessels by decreasing endothelin-1 and AT1-receptor and increasing nitric oxide (NO).[38] A lack of NO contributes to impaired endothelial function and platelet aggregation, and enhances leucocyte adhesion to the endothelium. Moreover statins are antithrombotic by decreasing plasminogen activator and antiinflammatoric by decreasing adhesion molecules. Statins may

reduce apoptosis and cellular death by inhibiting the farnelysation of small G proteins, specifically Ras p21.[38]

Different studies have investigated the relationship between lipid levels and the risk of vascular dementia. Many of them found an association with decreased levels of HDL. Zuliani et al.[29] found lower levels of HDL in 60 subjects with VaD compared with 54 controls. Kuriyama et al.[1] reported lower HDL levels in 43 VAD patients compared with controls and Muckle et al.[2] found lower HDL in five VaD subjects compared with twelve AD patients. Van Exel et al. found a significant association between decreased HDL levels and cognitive impairment.[39] Sacco et al. found in a study from Northern Manhattan, the same community as of our sample, that high HDL levels were related to a lower risk of stroke,[40] which indirectly supports our findings. The role of LDL remains controversial. As Klich-Raczka et al.[4] and Paragh et al.[33] we found an association between increased LDL levels and the risk of VaD in a former study.[14] Other studies did not observe an association.[39,41]

Contradictory results have also been reported in AD. Both increased and reduced levels of HDL[3,8,9] and LDL[6,8] have been observed to be associated with AD risk. Interestingly, besides Lesser et al.,[7] who observed an association between high cholesterol levels and the risk of AD, Scacchi et al.[6] and Kuusisto et al.[42] found an association between high cholesterol levels and a lower risk of AD.

The role of lipid lowering drugs also remains unclear. Besides Hajjar et al.,[11] who reported an association of lipid lowering agents with a lower risk of Alzheimer's disease and vascular dementia, Muldoon et al.[43] found a decrease in cognitive function in subjects using statins.

Our results are consistent with the studies by Zuliani et al.,[29] Kuriyama et al.[1] or Muckle et al.,[2] showing an association between low levels of HDL and the risk of VaD. They also agree with the findings by Paragh et al.[33] and Klich-Razka et al.[4] showing an association between the risk of vascular dementia and high LDL levels. Contrary to the results by Hajjar et al.[11] we did not find an association between the use of lipid lowering agents and the risk of vascular dementia. Unlike the study by Lesser et al.,[7] we did not observe an association between plasma lipids and the risk of AD. We found in the cross-sectional analysis a negative association between the use of statins and the risk of AD, as reported by Hajjar et al.,[11] but we did not replicate this in the longitudinal study. This discrepancy may be due to confounding by indication in the cross-sectional analyses. That is, persons with AD are not described statins, while this confounding does not occur in the longitudinal analysis.

In our study we observed an unexpected association between high cholesterol levels and a lower risk of AD. A possible explanation for this is the nutritional status of elderly in the early, prodromal stages of AD. At this stage patients show alterations in the energetic profile as

weight loss, reduced caloric intake and increased energy requirement,[6] and it is possible that low cholesterol levels reflect malnutrition in subjects with prodromal AD.

We found an association between higher LDL levels and a higher risk of VaD in the longitudinal study but not in the cross-sectional study. However, the results of the latter were close to statistical significance. We also observed an association between lower HDL levels and a higher risk of VaD in the cross-sectional but not longitudinal study. Since the sample size of the longitudinal study was much smaller, it could be considered that it lacked statistical power. However, in this study we had in both cross-sectional and prospective analysis 80% power to detect a relative risk of 2.0. Associations of smaller magnitude may be explained by bias and confounding.[44] In addition, regardless of the power of our data, our analyses with Alzheimer's disease as an outcome clearly show that the hazard ratios were close to one. The magnitude of the hazard ratios and the confidence intervals did not suggest an association between higher lipid levels and an increased risk of AD, making it unlikely that our analyses missed meaningful associations due to lack of power.

Compared with the lipid levels of the NHANES III population of similar age and gender levels of total cholesterol and LDL levels were slightly lower and triglyceride levels slightly higher in our population, while HDL levels were similar. However, it is important to point out that NHANES III data sampled Whites, Mexican Americans, and African Americans[45] while almost half of our sample is comprised of Caribbean Hispanics, who are not represented in NHANES. Thus, NHANES may not be generalizable to our urban sample from Northern Manhattan.

The main limitation of this study is that we had only one measurement of lipid levels, which could have led to measurement error and an underestimation of the association between lipid levels and dementia.

In summary, we found that the risk of VaD increases with lower HDL levels and higher levels of non-HDL cholesterol and LDL in cross-sectional or longitudinal analysis. Our results do not support the hypothesis that the risk of AD is associated with plasma lipid levels. They also do not support the hypothesis that statin use is associated with a lower risk of AD. The relation between HDL and VaD needs further exploration in a larger prospective study.

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2.2

Impact of plasma lipids and time on memory performance in healthy elderly without dementia

ABSTRACT

Objective. To examine the association of plasma lipid levels to changes in cognitive function in the elderly without dementia. **Methods.** We examined changes in performance in tests of memory, visuospatial/cognitive and language abilities in 1147 elderly individuals without dementia or cognitive impairment at baseline followed for seven years using generalized estimating equations. **Results.** Performance in all cognitive domains declined significantly over time, while there was no association between levels of any plasma lipid or lipid lowering treatment and memory, cognitive/visuospatial or language performance at any interval. Higher age at baseline was related to lower scores in all three domains at each interval, while higher education and Caucasian ethnicity were associated with higher scores in all domains. Analyses relating plasma lipids to performance in color trails tests using proportional hazards regression showed no association. In subsequent analyses excluding subjects with incident dementia, memory performance declined over time, while cognitive/visuospatial and language performance did not. Higher plasma HDL and total cholesterol were associated with higher scores in language performance at baseline; this domain declined faster among individuals with higher total cholesterol, but this result was not significant after taking multiple comparisons into account. Plasma triglycerides, LDL, or treatment with lipid lowering agents were not associated to changes in cognitive performance. **Conclusions.** Plasma lipid levels or treatment with lipid lowering agents in the elderly were not associated with changes in cognitive function.

INTRODUCTION

Dyslipidemia and dementia are among the most common diseases in western societies. About 1 percent of people aged 65-69 years develop dementia, and the prevalence increases to more than 60 percent for people over the age of 95.[1] More than 50 percent of the US population age 20 years or older suffer from cholesterol 200 mg/dl or higher, and more than 18 percent show cholesterol levels equal to or over 240 mg/dl.[2] There is conflicting data showing that dyslipidemia, a modifiable risk factor, is associated with a higher risk of cognitive impairment or dementia. Reduced high-density lipoprotein cholesterol (HDL-C)[3-9] and apolipoprotein A-1 levels,[3] as well as increased levels of lipoprotein (a)[5] have been observed in dementia in some but not all studies. There also have been contradictory results in studies relating total cholesterol[10,11] and low-density lipoprotein cholesterol (LDL-C)[6,8,11] to dementia.

Interest in these relationships has been increased by the observation that the use of widely available lipid lowering agents, HMG-COA-reductase-inhibitors (statins), may be associated with a lower risk of dementia.[12] In addition, cholesterol alters the degradation of the amyloid precursor protein (APP), which plays a major role in the pathogenesis of Alzheimer's disease (AD).[13] Moreover, vascular disease, which is associated with dyslipidemia, may be related to the risk of cognitive decline[14,15] and dementia. We previously reported an association between high levels of total and LDL-C and vascular dementia,[16] but no association between LDL-C and AD.

Our objective in this study was to examine the association between plasma lipid levels in the elderly and decline in memory and other cognitive functions.

METHODS

Subjects and Setting. Participants were enrolled in a longitudinal cohort study by a random sampling of Medicare recipients 65 years or older residing in northern Manhattan (Washington Heights, Hamilton Heights, Inwood). The sampling procedures have been described elsewhere.[17] Each participant underwent an in-person interview of general health and function at the time of study entry followed by a standard assessment, including medical history, physical and neurological examination as well as a neuropsychological battery.[18] Baseline data were collected from 1992 through 1994. Follow-up data were collected during evaluations at sequential intervals of approximately 18 months, performed from 1994 to 1996, 1996 to 1997, and 1997 to 1999. In this elderly population, some participants did not complete follow up at all intervals due to refusal to participate further, relocation or death. About one half of participants were evaluated at the third follow-up visit. This study was approved by the institutional review board of the Columbia-Presbyterian Medical Center.

The sample for this study were individuals with lipid levels obtained at the first follow-up interval, without dementia or cognitive impairment at baseline and the first interval, and with complete neuropsychological information in at least 3 follow-up intervals. Of the 2126 individuals who underwent clinical assessment at baseline, 327 individuals were excluded due to dementia at baseline. Plasma lipids were unavailable in 140 cases, and at first follow-up visit 94 subjects were excluded due to prevalent dementia, 104 subjects due to cognitive impairment without dementia (Clinical Dementia Rating Scale Score of 0.5),[19] and 141 subjects were dead, 117 refused to participate further and 56 were relocated (Figure 1).

Thus we restricted the sample for these analyses to 1147 individuals without dementia (AD or other forms), and without cognitive impairment without dementia, stroke, Parkinson's disease or other major neurological disorders at baseline or first follow-up interval. Subjects who developed cognitive impairment or dementia after the first follow-up visit were included in the main analyses.

Clinical assessments. Data included medical, neurological, and neuropsychological evaluations.[18,20] All participants underwent a standardized neuropsychological test battery that examined multiple domains in either English or Spanish.[18] Orientation was evaluated using parts of the modified Mini-Mental State Examination.[21] Language was assessed using the Boston Naming Test,[22] the Controlled Word Association Test,[23] category naming, and the Complex Ideational Material and Phrase Repetition subtests from the Boston Diagnostic Aphasia Evaluation.[24] Abstract Reasoning was evaluated using WAIS-R Similarities subtest,[25] and the

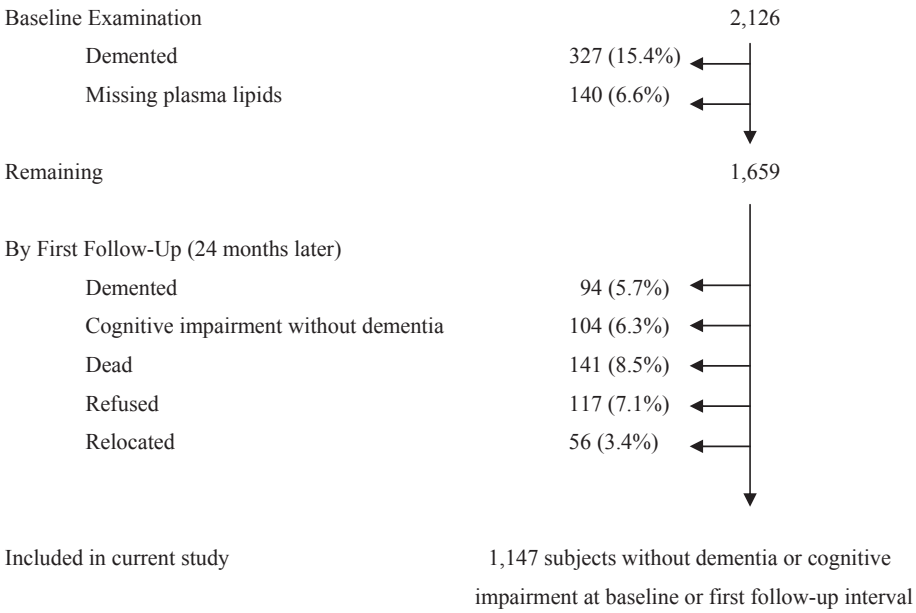

Figure 1. Description of Sample Size

non-verbal Identities and Oddities subtest of the Mattis Dementia Rating Scale.[26] Visuospatial ability was examined using the Rosen Drawing Test,[27] and a matching version of the Benton Visual Retention Test.[28] Memory was evaluated using the multiple choice version of the Benton Visual Retention Test[28] and the seven subtests of the Selective Reminding Test:[29] total recall, long-term recall, long-term storage, continuous long-term storage, words recalled on last trial, delayed recall, and delayed recognition. This neuropsychological test battery has established norms for the same community.[30] Results from the neurological, psychiatric and neuropsychological examinations were reviewed in a consensus conference comprised of physicians, neurologists, neuropsychologists and psychiatrists. Based on this review all participants were assigned to one of three categories: normal cognitive function, cognitive impairment without dementia, or dementia. Dementia was defined by DSM-IV criteria[31] and required cognitive impairment in several domains and functional impairment (Clinical Dementia Rating (CDR)≥1).[19] Cognitive impairment without dementia was diagnosed in participants who had abnormal results in cognitive tests, but had no significant cognitive impairment (CDR=0.5). Color trails were available only in the 1999 follow-up. Thus, analyses with the color trails were conducted separately only in individuals who were in the study beyond 1999 and had information on color trails (n=453). The color trails were not part of the calculated cognitive scores and were dichotomized for prospective analyses.

Plasma Lipids and APOE Genotyping. Fasting plasma total cholesterol and triglyceride levels were determined at the first follow-up interval using standard enzymatic techniques. HDL-C levels were determined after precipitation of apolipoprotein B containing lipoproteins with phosphotungstic acid.[32] LDL-C was recalculated using the formula of Friedewald et al.[33]

APOE genotypes were determined as described by Hixson and Vernier[34] with slight modification.[35] We classified persons as homozygeous or heterozygeous for the APOE ε4 allele or not having any ε4 allele.

Statistical Methods. A factor analysis was performed using data from all visits of the analytic sample with the 15 neuropsychological measures using a principal component analysis with varimax rotation and Kaiser normalization.[36] This analysis resulted in three factors: 1) a memory factor, in which the seven subtests of the Selective Reminding Test[29] were the main contributors; 2) a visuospatial/cognitive factor, where visuospatial and tests of reasoning were the main contributors; and 3) a language factor, in which language measures from the Boston Naming Test,[22] Controlled Oral Word Association Test,[23] and the WAIS-R Similarities[25] were the main contributors. We calculated cognitive scores for each participant at each visit by adding the scores of the measures that contributed most to each factor (tests with correlations of 0.5 or higher). Each factor score was normally distributed. These factors remained stable when we excluded subjects who developed dementia during follow-up, and were reproducible at baseline and at each follow-up interval.

Analysis of prospective change in the memory score was performed by applying generalized estimating equations (GEE)[37] with repeated measures. This statistical method takes into account the multiple observations per subject which are likely to be correlated, and treats them as clusters. The dependent variables were the calculated cognitive scores, and the independent variables were plasma lipid levels of total cholesterol, HDL-C, triglycerides and LDL-C, time (included as a continuous variable), and the interaction of plasma lipids and time. Plasma lipid levels were examined first dichotomized by the median and in subsequent models using the accepted limits of normal as cutoff points (240 mg/dl for total cholesterol, 40 mg/dl for HDL-C, 200 mg/dl for triglycerides and 160 mg/dl for LDL-C)[2]. Gender, age, education and ethnic group were included as covariates in subsequent analyses. Because the distribution of HDL-C and triglycerides was skewed, logarithmic transformation of these data was carried out before statistical tests were performed.

The GEE analysis yields coefficient values which represent the associations between a factor score and variables included in the model. There were three main coefficients of interest in each model: one comparing the lipid groups at baseline, one relating the change in cognitive scores with time, and an interaction term for time and lipid group. A significant p value for the coefficient comparing lipids at baseline indicates a difference between two groups at baseline. A significant p value for the coefficient of time indicates a statistically significant change in a cognitive score over the total duration of follow-up. A significant p value for the interaction coefficient indicates a difference in the rate of change in a factor score depending on the plasma lipid level; this is the main variable of interest for the interpretation of the analyses.

We also conducted analyses restricted to the subjects with data on color trails. We dichotomized the color trails time by the 75th percentile, and conducted proportional hazards models relating plasma lipid levels with poor performance in color trails, adjusting for gender and age, baseline cognitive scores, and other variables. The time-to-event variable was age-at-onset of low performance in color trails. Information on covariates was obtained at baseline. Data analysis was performed using SPSS version 12.0.

RESULTS

The mean age was 76.3 years, and 68.4% of the study population were women, 46.3% were Hispanic, 20.8% were White, and 32.3% were Black (Table 1). The mean of years of education was 8.6, and 27.5% were homozygous or heterozygous for the APOE-ε4 allele. The mean level of total cholesterol was 203.1, of HDL-C 47.1, of triglycerides 185.2 and of LDL-C 118.9 mg/dl. The mean body mass index was 27.1, and 15.8% of the subjects reported having diabetes, 50.3% hypertension and 14.7% heart disease. Use of lipid lowering agents was reported by 59 subjects (5.1%). There were 7217 person-years of follow-up, and the mean duration of follow-up was 5.6 ± 2.3 years.

Table 1. Demographic characteristics of the 1,147 individuals in the study population

Men	363 (31.6)
Women	784 (68.4)
Education, mean (SD), year	8.6 (4.6)
Age, mean (SD), year	76.3 (5.8)
Body mass index, mean (SD)	27.1 (5.1)
Ethnic group ‡	
White/Non-Hispanic	239 (20.8)
Black/Non-Hispanic	371 (32.3)
Hispanic	531 (46.3)
APOE genotype 4/4	22 (1.9)
APOE genotype 4/-	294 (25.6)
APOE genotype -/-	682 (71.6)
Cholesterol (mg/dl), mean (SD)	203.1 (40.7)
HDL (mg/dl), mean (SD)	47.1 (15.8)
Triglycerides (mg/dl), mean (SD)	185.2 (95.7)
LDL (mg/dl), mean (SD)	118.9 (36.4)
No Diabetes	852 (74.3)
Diabetes, not treated	47(3.6)
Diabetes, treated	140 (12.2)
No heart disease	871 (75.9)
Heart disease, not treated	41 (3.6)
Heart disease, treated	127 (11.1)
No hypertension	458 (39.9)
Hypertension, not treated	173 (15.1)
Hypertension, treated	404 (35.2)
Use of lipid lowering agents	
no	763 (66.5)
yes	59 (5.1)

Values are expressed as number (percentage) unless otherwise indicated. Some percentages are based on an incomplete sample due to small amounts of missing data. ‡ Classified by self-report using the format of the 1990 US census.

Women had higher levels of total cholesterol, HDL, triglycerides and LDL than men (Table 2). Hispanics had lower levels of total cholesterol, HDL and LDL, and higher levels of triglycerides than Whites and Blacks.

In the GEE analysis performance in all cognitive domains declined significantly over time, while there was no association between levels of any plasma lipid or lipid lowering treatment and memory, cognitive/visuospatial or language performance at any interval (Tables 3, 4 and 5). These results remained unchanged when not the median but accepted limits of normal were used as cutoff points for plasma lipid levels, or when analyses were stratified by APOEε4

Table 2. Comparison of lipid levels by demographics in 1,147 subjects

	Cholesterol (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)
Men	188.8 (39.2)	41.9 (12.5)	182.5 (103.4)	110.5 (35.8)
Women	209.7 (39.7) *	49.4 (16.5) *	186.5 (92.1)	122.8 (36.0) *
Ethnic group †				
White/Non-Hispanic	209.3 (40.4)*	47.4 (16.3) *	186.5 (94.3)	124.6 (33.3)*
Black/Non-Hispanic	203.4 (40.7)	51.1 (16.3) *	158.7 (78.1)	120.6 (37.4)*
Hispanic	199.9 (40.8)	43.8 (46.3)	203.8 (103.4)*	115.2 (36.9)

Values are expressed as number (SD) unless otherwise indicated. Some percentages are based on an incomplete sample due to small amounts of missing data. * Significant at a 0.05 level versus lowest value within lipid group, based on analysis of variance for continuous data and χ^2 test for categorical data. † Classified by self-report using the format of the 1990 US census.

Table 3. Relationship of plasma lipids and time of follow-up to memory performance in healthy elderly over 7 years

	Model 1		Model 2	
Variable	**Estimated ß (SE)**	**p-value**	**Estimated ß (SE)**	**p-value**
Time	-6.8 (0.6)	<0.0001	-6.8 (0.6)	<0.0001
Total cholesterol	3.5 (3.0)	0.3	-0.6 (2.8)	0.8
Time*total cholesterol	-0.4 (0.8)	0.6	-0.4 (0.8)	0.6
Time	-7.3 (0.6)	<0.0001	-7.3 (0.6)	<0.0001
HDL	-3.3 (3.0)	0.3	-1.2 (2.8)	0.7
Time*HDL	0.5 (0.8)	0.5	0.6 (0.8)	0.5
Time	-6.9 (0.6)	<0.0001	-6.9 (0.6)	<0.0001
Triglycerides	0.5 (3.0)	0.9	2.3 (2.8)	0.4
Time*triglycerides	-0.3 (0.8)	0.7	-0.1 (0.8)	0.9
Time	-7.1 (0.6)	<0.0001	-6.9 (0.6)	<0.0001
LDL	1.2 (3.0)	0.7	-0.9 (2.8)	0.7
Time*LDL	0.2 (0.8)	0.8	-0.1 (0.8)	0.9

Model 1 is adjusted for age and gender, Model 2 is adjusted for age, gender, education, ethnic group and APOEε4

genotype or ethnic group. Higher age at baseline was related to lower scores in all three domains at each interval, while higher education and Caucasian ethnicity were associated with higher scores in all domains.

Cox proportional hazards analysis relating plasma lipid levels and the incidence of low performance in color trail tasks also showed no association (total cholesterol: HR 1.0, 95% CI 0.9-1.1; HDL-C: HR 0.9, 95% CI 0.9-1.1, triglycerides: 1.1, 95% CI 0.9-1.0; LDL-C: HR 1.0, 95% CI 0.9-1.0).

Table 4. Relationship of plasma lipids and time of follow-up to cognitive performance in healthy elderly over 7 years

	Model 1		Model 2	
Variable	**Estimated ß (SE)**	**p-value**	**Estimated ß (SE)**	**p-value**
Time	-1.0 (0.2)	<0.0001	-1.0 (0.2)	<0.0001
Total cholesterol	1.4 (1.5)	0.4	-1.7 (1.2)	0.1
Time*total cholesterol	0.3 (0.3)	0.4	0.2 (0.3)	0.4
Time	-0.9 (0.2)	<0.0001	-0.9 (0.2)	<0.0001
HDL	-1.9 (1.6)	0.2	0.1 (1.3)	0.9
Time*HDL	-0.1 (0.3)	0.6	-0.1 (0.3)	0.8
Time	-0.9 (0.2)	<0.0001	-0.9 (0.2)	<0.0001
Triglycerides	-2.3 (1.5)	0.1	-0.1 (1.2)	0.9
Time*triglycerides	0.0 (0.3)	0.9	0.2 (0.3)	0.5
Time	-1.2 (0.2)	<0.0001	-1.1 (0.2)	<0.0001
LDL	-0.1 (1.5)	0.9	-1.8 (1.2)	0.1
Time*LDL	0.5 (0.3)	0.06	0.4 (0.3)	0.2

Model 1 is adjusted for age and gender, Model 2 is adjusted for age, gender, education, ethnic group and APOEε4

Table 5. Relationship of plasma lipids and time of follow-up to language performance in healthy elderly over 7 years

	Model 1		Model 2	
Variable	**Estimated ß (SE)**	**p-value**	**Estimated ß (SE)**	**p-value**
Time	-0.2 (0.1)	<0.0001	-0.2 (0.1)	<0.0001
Total cholesterol	0.2 (0.3)	0.4	0.1 (0.3)	0.7
Time*total cholesterol	-0.1 (0.1)	0.7	-0.1 (0.1)	0.6
Time	-0.2 (0.1)	<0.0001	-0.2 (0.1)	0.002
HDL	-0.3 (0.3)	0.3	-0.2 (0.3)	0.5
Time*HDL	0.0 (0.1)	0.6	0.1 (0.1)	0.5
Time	-0.2 (0.1)	0.005	-0.3 (0.1)	<0.0001
Triglycerides	-0.1 (0.3)	0.9	0.2 (0.3)	0.5
Time*triglycerides	0.1 (0.1)	0.4	0.1 (0.1)	0.1
Time	-0.2 (0.1)	0.006	-0.2 (0.1)	0.002
LDL	0.1 (0.3)	0.9	0.1 (0.3)	0.9
Time*LDL	0.1 (0.1)	0.4	0.1 (0.1)	0.5

Model 1 is adjusted for age and gender, Model 2 is adjusted for age, gender, education, ethnic group and APOEε4

In subsequent analyses we excluded subjects who developed dementia during follow-up (n=198). While memory performance declined significantly over time, cognitive/visuospatial and language performance did not change. There was no association between plasma levels of triglycerides or LDL and performance on any of the three cognitive factors at any time interval. While increased levels of total cholesterol and HDL-C were associated with higher scores in language performance, there was a statistically significant total cholesterol*time (duration of follow-up) interaction indicating that language performance declined at a faster rate among individuals with higher total cholesterol levels compared to subjects with lower levels. This association remained significant after adjusting for age, gender, ethnic group, education and APOE allele. However, this association was not significant considering Bonferroni correction for multiple comparisons.[38] There was no similar relationship between total cholesterol*time effect and memory or visuospatial/cognitive factors. However, scores of both factors were normally distributed at each time interval indicating that the lack of a total cholesterol*time interaction was not the result of a ceiling or floor effect.

Treatment with lipid lowering agents was not associated with better scores on any of the three cognitive factors at any time interval, and cox proportional hazards analysis relating plasma lipid levels and the incidence of low performance in color trail tasks also showed no association (total cholesterol: HR 0.9, 95% CI 0.9-1.0; HDL-C: HR 1.2, 95% CI 0.7-42.2, triglycerides: 1.0, 95% CI 0.2-4.2; LDL-C: HR 0.9, 95% CI 0.9-1.0).

DISCUSSION

In this study performance in all cognitive domains declined significantly over time in elderly individuals without dementia or cognitive impairment, while there was no association between levels of any plasma lipid or lipid lowering treatment and memory, cognitive/visuospatial or language performance at any interval. Higher age at baseline was related to lower scores in all three domains at each interval, while higher education and Caucasian ethnicity were associated with higher scores in all domains.

The role of dyslipidemia in the development of cognitive impairment remains unclear. Brain cholesterol alters the degradation of APP(2), which contributes to the pathogenesis of AD.[13] Several lines of evidence indicate that lowering plasma cholesterol levels prevents AD development by reducing Aβ production and secretion.[39] These findings seem to contradict previous studies demonstrating that cholesterol protects PC12 cells from fibrillar Aβ peptide, that cholesterol depletion induces AD-type injuries in cultured hippocampal slices,[39] and that brain cholesterol is almost entirely synthesized in situ and not transferred from the plasma into the brain.[40] Few studies have examined the association of plasma lipid levels to cognitive function, and they reported inconsistent results.[5,7,41-43] Results in animal studies,[44,45] and

studies relating plasma lipid lowering treatment to cognitive functioning[7,12,42,46,47] have also been conflicting. Most observational studies were cross-sectional,[9,42,48-50] and some of the few longitudinal studies included individuals with questionable dementia or AD and did not provide methods to limit inclusion of such individuals.[51] Our results are consistent with the idea that plasma lipid levels do not affect cognition directly.

There are several potential explanations for our findings of no association of plasma lipids and lipid lowering treatment to cognitive change. One explanation is measurement error. We had only one measure of plasma lipids which may not take into account intrapersonal variation. If the measurement error was random, this would have underestimated the association between lipids and cognitive changes, thus resulting in finding of no association. Another possibility is that our sample was relatively homogeneous in plasma lipid levels, thus not permitting enough variability to detect an association. Another potential explanation is bias related to selection into this study. It is possible that plasma lipid levels are related to cognitive decline in younger individuals but not the older sample in our study. Our sample was older than 65 years with a mean age of 75.7 years. It is possible that individuals with adverse outcomes related to plasma lipid levels did not survive to inclusion in our study, or that the plasma lipid levels at the age of entry in the study did not reflect lipid levels earlier in life. Finally, it is possible that plasma lipid levels are not related to cognitive decline as indicated by our results.

The main limitation of this study is that we used only one measurement of lipid levels, which could have led to measurement error due to intraperson variability and underestimation of the association between lipid levels and cognitive impairment.

This study has important strengths. This is a prospective cohort study designed for the diagnosis of cognitive decline, and with complete clinical and neuropsychological evaluation at each interval. Our study has sensitive measures of cognitive change in several specific domains including memory. In addition, we had the ability to diagnose dementia and cognitive impairment without dementia at baseline, thus allowing us to follow an unbiased sample. Other longitudinal studies used global cognitive assessments or may not have had the ability to detect early stages of cognitive impairment at baseline.[42,51,52]

An important consideration in the interpretation of the results of this study is its generalizability. This study was conducted in an urban multiethnic elderly community with a high prevalence of risk factors for mortality and dementia. Thus, our results may not be generalizable to cohorts with younger individuals or to cohorts with participants with a lower morbidity burden.

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2.3

Plasma Lipid Levels and Risk of Mild Cognitive Impairment

ABSTRACT

Objective. There are conflicting data relating plasma lipids to the risk of Alzheimer's disease (AD). In this study we explored the association of plasma lipid levels to mild cognitive impairment (MCI), a transitional stage between normal cognition and dementia. **Design and Setting.** Prospective community-based cohort study conducted in northern Manhattan. **Methods.** Multivariate proportional hazards regression analyses, relating plasma lipid levels to incident all-cause MCI, amnestic MCI, and non-amnestic MCI in 854 persons without prevalent MCI or dementia at baseline followed for a mean of 4.9 years. **Results.** There were 324 cases of incident MCI, 153 cases of amnestic MCI and 171 cases of non-amnestic MCI during 4189 person years of follow-up. Higher levels of total cholesterol and LDL were associated with a decreased risk of all-cause MCI. However, these associations were not statistically significant after adjusting for ethnic group, education, APOEε4 allele and other vascular risk factors. There was no independent association between lipids and the risk of amnestic MCI or non-amnestic MCI, and there was no effect on MCI risk by treatment with lipid lowering agents. **Conclusions.** Plasma lipid levels or treatment with lipid lowering agents in the elderly are not associated with the risk of all-cause MCI or MCI subtypes.

INTRODUCTION

Mild cognitive impairment (MCI), considered an intermediary stage between normal cognition and dementia, has become the focus of intense research interest, as a target of early detection and prevention of AD. MCI is defined by the presence of amnestic complaints, objective evidence of memory impairment, and absence of functional impairment.[1] The incidence rate of MCI among nondemented elderly is one percent per year,[2] but persons with MCI convert to AD at an annual rate of 10% to 12% in contrast to 1% to 2% in the elderly population without MCI.[3]

Dyslipidemia, a modifiable risk factor with a prevalence of more than 50% in persons age 20 years or older,[4] is associated with a higher risk of cognitive impairment or dementia. Reduced high-density lipoprotein cholesterol (HDL-C)[5-9] and apolipoprotein A-1 levels,[7] as well as increased levels of lipoprotein (a)[6] have been observed inconsistently in persons with dementia. There also have been contradictory results in studies relating cholesterol[10,11] and low-density lipoprotein cholesterol (LDL-C)[5,11] to dementia. Notably, cholesterol alters the degradation of the amyloid precursor protein (APP), which plays a major role in the pathogenesis of Alzheimer's disease (AD),[12] and the use of lipid lowering agents (statins), may be associated with a lower risk of dementia.[13] We previously reported associations between high levels of LDL-C and decreased levels of HDL-C and vascular dementia,[14] but no association between LDL-C and AD, or between plasma lipids and cognitive test performance over time.[15]

The objective in the present longitudinal study was to determine whether plasma lipid levels are associated with the risk of incident all-cause MCI, or amnestic or non-amnestic forms of MCI in the elderly.

METHODS

Subjects and Setting. Participants were enrolled in a longitudinal cohort study by a random sampling of Medicare recipients 65 years or older residing in northern Manhattan (Washington Heights, Hamilton Heights, Inwood). The sampling procedures have been described elsewhere.[16] Each participant underwent an in-person interview of general health and function at the time of study entry followed by a standard assessment, including medical history, physical and neurological examination as well as a neuropsychological battery.[17] Baseline data were collected from 1992 through 1994. Follow-up data were collected during evaluations at sequential intervals of approximately 18 months, performed from 1994 to 1996, 1996 to 1997, and 1997 to 1999. In this elderly population, some participants did not complete follow up at all intervals due to refusal to participate further, relocation or death. About one half of participants were evaluated at the third follow-up visit. This study was approved by the institutional review board of the Columbia-Presbyterian Medical Center.

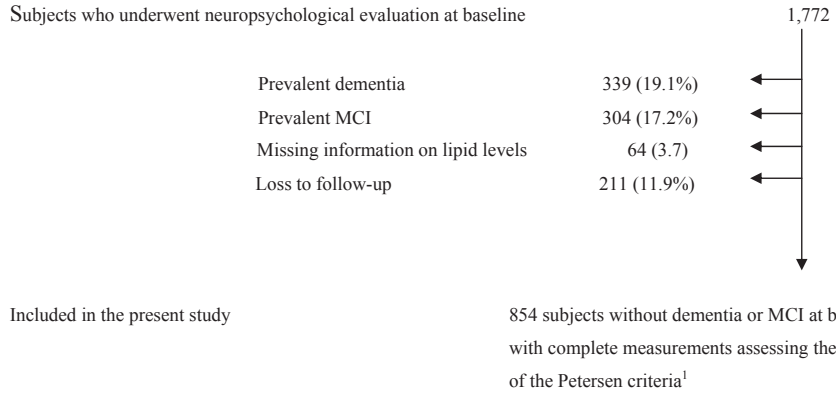

Figure 1. Description of sample size

Participants were without prevalent MCI or dementia at baseline, with information on plasma lipids, at least one follow-up interval, and with sufficient clinical information to ascertain MCI by the Petersen criteria.[1,3] Of the 1,772 participants in whom a full neuropsychological exam was attempted, 339 (19.1%) were excluded due to prevalent dementia, 304 (17.2%) were excluded due to prevalent MCI, 64 (3.7%) due to missing lipid levels, and 211 (11.9%) were excluded due to loss to follow-up (Figure 1). Thus, the final analytic sample included 854 individuals. Compared to the original sample the final sample at study baseline was younger (mean age 75.8 vs. 77.3; p <0.001), but had a similar proportion of women (69.0 vs. 69.4%), Blacks (33.0 vs 32.5%), Hispanics (44.8 vs. 47.0%), and Whites (22.2% vs. 20.4%).

Clinical assessments. Data were available from medical, neurological, and neuropsychological evaluations.[17,18] All participants underwent a standardized neuropsychological test battery that examined multiple domains in either English or Spanish at baseline and on subsequent assessments[17] using the Mini-Mental State Examination,[19] the Boston Naming Test,[20] the Controlled Word Association Test,[21] category naming, the Complex Ideational Material and Phrase Repetition subtests from the Boston Diagnostic Aphasia Evaluation,[22] the WAIS-R Similarities subtest,[23] the Mattis Dementia Rating Scale,[24] the Rosen Drawing Test,[25] the Benton Visual Retention Test,[26] the multiple choice version of the Benton Visual Retention Test[26] and the Selective Reminding Test.[27] This neuropsychological test battery has established norms for the same community.[28]

Diagnosis of Dementia. Diagnosis of dementia and assignment of specific cause was made by consensus of neurologists, psychiatrists, and neuropsychologists based on baseline and follow-up information. The diagnosis of dementia was based on DSM-IV criteria[29] and required evidence of cognitive deficits on the neuropsychological test battery as well as evidence of impairment in social or occupational function (Clinical Dementia Rating of 1 or more).[30] Diagnosis of AD was based on the NINCDS-ADRDA criteria.[31]

Definition of MCI. MCI criteria were retrospectively applied among nondemented individuals after the consensus conference. Consistent with standard criteria[1,3] for all subtypes of MCI, those considered for MCI were required to have: 1) a memory complaint 2) objective impairment in at least one cognitive domain based on the average of the scores on the neuropsychological measures within that domain and a 1.5 SD cutoff using normative corrections for age, years of education, ethnicity, and sex, 3) essentially preserved activities of daily living (defined above), and 4) no evidence of dementia.

The Petersen criteria,[1] which focus on memory impairment, were expanded to include mutually exclusive subtypes based on cognitive features. MCI-Amnestic (MCI-A), corresponds most closely to the original Petersen definition, and was defined as a memory score < 1.5 SD below demographically corrected mean on an average composite of the following measures: 1) total recall from the SRT 2) delayed free recall from the SRT, and 3) recognition from the BVRT. Performance on composite scores from all other cognitive domains was required to be within normal limits. Other MCI subtypes were classified that allowed for impairment in a single non-memory domain if performance on composite scores from all other cognitive domains was within normal limits. MCI-Executive Function (MCI-E) was defined by an average composite measure comprising the following measures: 1) Letter Fluency; 2) Category Fluency, and 3) the WAIS-R Similarities subtest. MCI-Language (MCI-L) was defined as isolated impairment on an average composite measure comprising: 1) Boston Naming Test; 2) BDAE Repetition, and the 3) BDAE Comprehension test. MCI-Visuospatial (MCI-V) was assigned if impairment was demonstrated on an average composite score comprising: 1) Rosen Drawing and 2) BVRT matching. Finally, we allowed for impairment in multiple cognitive domains in the absence of dementia. MCI-Multiple Cognitive Domains with memory impairment (MCI-MCDM) was defined by objective impairment on the memory domain composite score and if there was impairment on at least one other cognitive domain. MCI-Multiple Cognitive Domains without memory impairment (MCI-MCDN) was assigned if there was impairment in two or more of the three non-memory domains, and if the memory domain composite score was within normal limits. Again, classification into the six subtypes was mutually exclusive. We used three outcomes for these analyses: 1) all-cause MCI; 2) amnestic MCI, which included MCI-A and MCI-MCDM; and 3) non-amnestic MCI. The rationale for grouping MCI-A and MCI-MCDM is that they equally predict the development of AD in our cohort, and that MCI-MCDM is thought to be a more advanced form of MCI-A involving other cognitive domains.

Lipids and other covariates. Fasting plasma total cholesterol and triglyceride levels were determined at the first follow-up interval using standard enzymatic techniques. HDL-C levels were determined after precipitation of apolipoprotein B containing lipoproteins with phosphotungstic acid.[32] LDL-C was recalculated using the formula of Friedewald et al.[33]

At baseline, all participants were asked whether or not they had a history of hypertension at any time during their life. If affirmative, they were asked whether or not they were

under treatment and the specific type of treatment. Stroke was defined according to the WHO criteria.[34] The presence of stroke was ascertained from an interview with participants and their informants. Persons with stroke were confirmed through their medical records, 85% of which included results of brain imaging. The remainder was confirmed by direct examination. Diabetes mellitus was defined as a history at any time during life. At baseline, all participants were asked whether or not they had a history of diabetes. If affirmed, they were asked whether or not they were under treatment and the specific type of medication. Heart disease was defined as a history of atrial fibrillation and other arrhythmias, myocardial infarction, congestive heart failure or angina pectoris at any time during life.

APOE Genotyping. APOE genotypes were determined as described by Hixson and Vernier with slight modification.[35] We classified persons as homozygeous or heterozygeous for the APOEε4 allele or not having any ε4 allele.

Statistical Methods. First we evaluated plasma lipid levels, demographic distributions and clinical characteristics. We then used multivariate Cox proportional hazard models[36] to estimate the association of plasma lipid levels to incident all-cause MCI, amnestic MCI and non-amnestic MCI. Plasma lipids were analyzed first as continuous variables and later grouped into quartiles. Because the distribution of HDL-C and triglycerides was skewed, logarithmic transformation of these variables was carried out and the analyses repeated. The time-to-event variable was age at onset of MCI. Among individuals who did not develop MCI, those who developed dementia were censored at the time of dementia diagnosis, and those who did not develop dementia, who died, or who were lost to follow-up owing to relocation before development of MCI were censored at the time of their last evaluation. Information on covariates was obtained at baseline. After adjusting for sex and age, we additionally adjusted for ethnic group, education, APOE, diabetes, heart disease and hypertension in subsequent analyses. We performed all data analysis using SPSS version 13.0 software (SPSS Inc, Chicago, Ill).

RESULTS

There were 324 cases of incident MCI, 153 cases of amnestic MCI and 171 cases of non-amnestic MCI during 4189 person years of follow-up. This corresponds to incidence rates of 7.7% for MCI, 3.7% for amnestic MCI and 4.1% for non-amnestic MCI per person-year. The mean age of the sample was 75.8 ± 5.9 years, and 69.0% were women, 22.2% were non-Hispanic White, 33.0% non-Hispanic Black and 44.8% were Hispanic. The incidence rates of MCI, amnestic MCI and non-amnestic MCI did not significantly differ among the ethnic groups or sexes. The mean of years of education was 8.9 ± 4.5, and 68.6% had hypertension, 23.5% diabetes, and 34.1%

Table 1. Comparison of demographic and clinical characteristics by MCI status in 854 subjects

	No MCI (n=530)	Incident all-cause MCI (n=324)	Incident amnestic MCI (n=153)	Incident non-amnestic MCI (n=171)
Women, n (%)	364 (68.7)	225 (69.4)	106 (69.3)	119 (69.6)
Age, mean (SD), year	75.5 (6.0)	76.3 (5.7)	76.9 (5.7)	75.7 (5.7)
Education, mean (SD), year	9.1 (4.5)	8.6 (4.6)	9.1 (4.5)	8.3 (4.7)
Ethnic group, n (%) †				
White/Non-Hispanic	115 (21.7)	69 (21.3)	39 (25.5)	30 (17.5)
Black/Non-Hispanic	176 (33.2)	106 (32.7)	50 (32.7)	56 (32.7)
Hispanic	233 (44.0)	149 (46.0)	64 (41.8)	85 (49.7)
APOE genotype 4/- or 4/4, n (%)	144 (27.2)	96 (29.7)	49 (32.0)	47 (27.5)
Total cholesterol (mg/dl), mean (SD)	200.6 (41.2)	195.5 (40.9)	194.1 (39.4)	196.7 (42.4)
HDL-C (mg/dl), mean (SD)	47.6 (15.8)	46.9 (15.5)	47.7 (15.9)	46.2 (15.1)
Triglycerides (mg/dl), mean (SD)	163.5 (88.5)	157.5 (87.5)	149.6 (72.2)	164.7 (99.1)
LDL-C (mg/dl), mean (SD)	120.2 (36.4)	116.6 (34.3)	116.5 (33.6)	116.7 (35.1)
Stroke, n (%)	76 (14.3)	50 (15.4)	26 (17.0)	24 (14.0)
Diabetes, n (%)	112 (21.1)	89 (27.5)*	41 (26.8)	48 (28.1)*
Hypertension, n (%)	336 (63.4)	250 (77.2)*	113 (73.9)*	137 (80.1)*
Heart disease, n (%)	180 (34.0)	111 (34.3)	53 (34.6)	58 (33.9)
Current Smoking, n (%)	61 (11.5)	32 (9.9)	16 (10.5)	16 (9.4)
Lipid lowering treatment, n (%)	65 (12.3)	53 (16.4)	25 (16.3)	28 (16.4)

Some percentages are based on an incomplete sample due to small amounts of missing data. † Classified by self-report using the format of the 1990 US census.[60] MCI = mild cognitive impairment. HDL-C= high-density lipoprotein (HDL) cholesterol. LDL-C = low-density lipoprotein (LDL) cholesterol. * significant at a 0.05 level vs. No-MCI-group

heart disease. 28.1% of the sample were homo- or heterozygous for the APOEε4 allele. Use of lipid lowering medication was reported by 118 subjects (13.8%). Persons who developed all-cause MCI or non-amnestic MCI during follow-up had at baseline a higher prevalence of diabetes and hypertension than persons remaining free of MCI, and persons developing amnestic MCI reported more often a history of hypertension (table 1). Hispanics had at baseline slightly lower levels of HDL-C and higher levels of triglycerides compared with non-Hispanic Blacks and non-Hispanic Whites.

Plasma lipid levels and the risk of incident MCI. The mean age at onset of MCI was 80.7 ± 5.8 years. In multivariate analyses of the whole sample, higher plasma levels of total cholesterol and LDL-C were associated with a decreased risk of all-cause MCI after adjusting for age and sex (p for trend across quartiles of plasma lipids = 0.04 and 0.06, respectively; table 2). These associations were attenuated and became non-significant after additionally adjusting for ethnic group, education, APOEε4 allele, diabetes, heart disease and hypertension. There was no relation between lipids and the risk of amnestic MCI (table 3), and there was no association

Table 2. Hazard ratios and 95% confidence intervals, relating quartiles of plasma lipid levels and the risk of incident all-cause MCI

Quartiles range (mg/dl)	No. (%) of incident MCI	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Cholesterol			
1 (≤ 171.00)	88 (26.3)	1.0	1.0
2 (171.01-197.00)	96 (28.7)	1.1 (0.81-1.45)	1.1 (0.84-1.51)
3 (197.01-223.25)	73 (21.9)	0.8 (0.59-1.11)	0.8 (0.61-1.15)
4 (≥ 223.26)	77 (23.1)	0.7 (0.57-1.06)	0.8 (0.58-1.11)
trend test		p=0.04	p=0.08
HDL-C			
1 (≤ 37.00)	88 (26.3)	1.0	1.0
2 (37.01-45.00)	66 (19.8)	0.8 (0.59-1.12)	0.8 (0.61-1.16)
3 (45.01-55.00)	77 (23.1)	0.9 (0.63-1.17)	0.9 (0.65-1.23)
4 (≥ 55.01)	103 (30.8)	0.8 (0.61-1.09)	0.9 (0.64-1.17)
trend test		p=0.2	p=0.4
Triglycerides			
1 (≤ 98.25)	92 (27.5)	1.0	1.0
2 (98.26-142.50)	81 (24.3)	0.9 (0.65-1.18)	0.9 (0.65-1.19)
3 (142.51-197.50)	72 (21.6)	0.8 (0.58-1.03)	0.7 (0.53-1.01)
4 (≥ 197.51)	89 (26.6)	0.9 (0.72-1.28)	0.9 (0.65-1.21)
trend test		p=0.6	p=0.3
LDL-C			
1 (≤ 95.40)	90 (26.9)	1.0	1.0
2 (95.41-116.00)	80 (24.0)	0.8 (0.61-1.12)	0.9 (0.63-1.15)
3 (116.01-141.75)	84 (25.1)	0.8 (0.63-1.14)	0.9 (0.67-1.21)
4 (≥ 141.76)	80 (24.0)	0.7 (0.54-0.99)*	0.8 (0.56-1.04)
trend test		p=0.06	p=0.1

Model 1: adjusted for sex and age. Model 2: adjusted for sex, age, ethnic group, education, APOE, diabetes, heart disease and hypertension. MCI = mild cognitive impairment. HDL-C= high-density lipoprotein (HDL) cholesterol. LDL-C = low-density lipoprotein (LDL) cholesterol

between lipids and non-amnestic MCI in either model (table 4). Treatment with lipid lowering agents was not associated with the risk of all-cause MCI (HR 1.0, 95% CI 0.75-1.37), amnestic MCI (HR 1.0, 95% CI 0.66-1.58), or non-amnestic MCI (HR 1.0, 95% CI 0.67-1.52).

There was no association between lipids and MCI, amnestic MCI or non-amnestic MCI in analyses restricted to persons with longer-follow up time (observation time ≥ the median follow-up time of 3.9 years), or in analyses stratified by median of age (74.7 years). There was also no association in analyses stratified by ethnic group.

Table 3. Hazard ratios and 95% confidence intervals, relating quartiles of plasma lipid levels and the risk of incident amnestic MCI

Quartiles range (mg/dl)	No. (%) of incident amnestic MCI	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Cholesterol			
1 (≤ 171.00)	48 (30.0)	1.0	1.0
2 (171.01-197.00)	39 (24.4)	0.8 (0.54-1.25)	0.8 (0.53-1.25)
3 (197.01-223.25)	35 (21.9)	0.7 (0.45-1.11)	0.7 (0.44-1.09)
4 (≥ 223.26)	38 (23.8)	0.7 (0.46-1.09)	0.6 (0.40-1.02)
trend test		p=0.09	p=0.04
HDL-C			
1 (≤ 37.00)	37 (23.1)	1.0	1.0
2 (37.01-45.00)	30 (18.8)	0.9 (0.53-1.41)	0.9 (0.55-1.44)
3 (45.01-55.00)	41 (25.6)	1.1 (0.69-1.71)	1.1 (0.71-1.78)
4 (≥ 55.01)	52 (32.5)	1.0 (0.64-1.51)	1.0 (0.62-1.53)
trend test		p=0.8	p=0.9
Triglycerides			
1 (≤ 98.25)	46 (28.8)	1.0	1.0
2 (98.26-142.50)	38 (23.8)	0.8 (0.54-1.27)	0.8 (0.55-1.31)
3 (142.51-197.50)	36 (22.5)	0.8 (0.49-1.19)	0.8 (0.48-1.17)
4 (≥ 197.51)	40 (25.0)	0.9 (0.57-1.34)	0.8 (0.51-1.26)
trend test		p=0.5	p=0.3
LDL-C			
1 (≤ 95.40)	40 (25.0)	1.0	1.0
2 (95.41-116.00)	39 (24.4)	0.9 (0.59-1.43)	0.9 (0.56-1.38)
3 (116.01-141.75)	43 (26.9)	1.0 (0.63-1.48)	1.0 (0.61-1.47)
4 (≥ 141.76)	38 (23.8)	0.8 (0.49-1.21)	0.7 (0.41-1.09)
trend test		p=0.3	p=0.1

Model 1: adjusted for sex and age. Model 2: adjusted for sex, age, ethnic group, education, APOE, diabetes, heart disease and hypertension. MCI = mild cognitive impairment. HDL-C= high-density lipoprotein (HDL) cholesterol. LDL-C = low-density lipoprotein (LDL) cholesterol

DISCUSSION

In this longitudinal analysis of 854 persons, higher plasma levels of total cholesterol and LDL-C were associated with a lower risk of all-cause MCI in analyses for the whole sample after adjusting for age and sex. Both associations were attenuated after additionally adjusting for ethnic group, education and potential vascular risk factors. There was no relation between lipid levels and the risk of amnestic MCI or non-amnestic MCI.

Table 4. Hazard ratios and 95% confidence intervals, relating quartiles of plasma lipid levels and the risk of incident non-amnestic MCI

Quartiles range (mg/dl)	No. (%) of incident non-amnestic MCI	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Cholesterol			
1 (≤ 171.00)	40 (23.0)	1.0	1.0
2 (171.01-197.00)	57 (32.8)	1.4 (0.93-2.09)	1.5 (0.99-2.25)
3 (197.01-223.25)	38 (21.8)	0.9 (0.60-1.47)	1.1 (0.64-1.59)
4 (≥ 223.26)	39 (22.4)	0.9 (0.55-1.34)	0.9 (0.61-1.56)
trend test		p=0.2	p=0.5
HDL-C			
1 (≤ 37.00)	51 (29.3)	1.0	1.0
2 (37.01-45.00)	36 (20.7)	0.8 (0.50-1.19)	0.8 (0.53-1.25)
3 (45.01-55.00)	36 (20.7)	0.7 (0.46-1.06)	0.8 (0.50-1.21)
4 (≥ 55.01)	51 (29.3)	0.7 (0.47-1.04)	0.8 (0.53-1.21)
trend test		p=0.07	p=0.3
Triglycerides			
1 (≤ 98.25)	46 (26.4)	1.0	1.0
2 (98.26-142.50)	43 (24.7)	0.9 (0.60-1.39)	0.9 (0.58-1.35)
3 (142.51-197.50)	36 (20.7)	0.7 (0.48-1.16)	0.8 (0.44-1.07)
4 (≥ 197.51)	49 (28.2)	1.0 (0.69-1.56)	0.9 (0.64-1.49)
trend test		p=0.9	p=0.6
LDL-C			
1 (≤ 95.40)	50 (28.7)	1.0	1.0
2 (95.41-116.00)	41 (23.6)	0.8 (0.50-1.15)	0.8 (0.51-1.20)
3 (116.01-141.75)	41 (23.6)	0.8 (0.49-1.14)	0.8 (0.54-1.24)
4 (≥ 141.76)	42 (24.1)	0.7 (0.46-1.04)	0.8 (0.49-1.18)
trend test		p=0.09	p=0.3

Model 1: adjusted for sex and age. Model 2: adjusted for sex, age, ethnic group, education, APOE, diabetes, heart disease and hypertension. MCI = mild cognitive impairment. HDL-C= high-density lipoprotein (HDL) cholesterol. LDL-C = low-density lipoprotein (LDL) cholesterol

The role of dyslipidemia in the pathogenesis of cognitive impairment remains controversial. Brain cholesterol alters the degradation of APP(2), which in turn promotes the pathogenesis of AD.[12] Several reports indicate that lowering plasma cholesterol levels prevents AD development by reducing Aβ production and secretion.[37] However, there are contradictory studies demonstrating that cholesterol protects PC12 cells from fibrillar Aβ peptide and that cholesterol depletion induces AD-type injuries in cultured hippocampal slices.[37] There is also evidence that plasma cholesterol levels have no effect on brain HMG-CoA-reductase levels and its activity[8] or levels of 24S-hydroxycholesterol, which is a degradation product of brain cholesterol.[38] Statins might decrease the risk of cognitive impairment by having a lowering effect on plasma lipid levels, or by their inflammatory or pleiotropic effects.[39]

Studies examining the role of plasma lipid levels in cognitive function reported inconsistent results.[6,8,40,41] Controversial results have also been obtained in animal studies,[42,43] and studies relating plasma lipid lowering treatments to cognitive function.[8,13,41] Most observational studies were cross-sectional,[44,45] and the few longitudinal studies mostly examined manifest dementia but not MCI as the clinical endpoint.[46,47] While studies have found a relation between high cholesterol during mid-life and cognitive impairment or MCI in old age,[48] similar associations relating late-life lipids with cognitive impairment or dementia have not been observed.

We found no relation between HDL-C or triglyceride levels and all-cause MCI, amnestic MCI or non-amnestic MCI. This is consistent with our previous observations of no association between lipid levels and cognitive performance in several domains over time,[15] as well as other cross-sectional and longitudinal studies observing no relation between plasma lipids and cognitive impairment or dementia.[6,49,50]

We found no association between lipid lowering treatments and MCI. Case-control studies have suggested that statin use is associated with a significant decrease in prevalence of AD or dementia.[51-53] However, several recent prospective cohort studies were inconclusive.[54-56] Interestingly, in two of the studies, the researchers found an association with decreased prevalence of AD or dementia in current statin users, but no association with incidence within the same cohorts[55,56] implying that cross-sectional and retrospective designs may have suffered from indication or selection bias.

We initially observed that higher cholesterol and LDL-C was related to a decreased risk of all-cause MCI after adjusting for age and sex, consistent with our previous observations showing relations between high cholesterol and a lower AD risk.[14] It is also consistent with other studies demonstrating a protective effect of late-life total cholesterol on the risk of MCI or AD.[11,57-59] However, as described above, there are contradictory studies reporting relations between elevated midlife total cholesterol levels (>6.5 mmol/L) and an increased risk of MCI.[48]

In this study, the association between higher cholesterol and LDL-C and risk of all cause MCI was attenuated after further adjustment with vascular risk factors.

It is possible that this inverse relationship is due to a survival bias, that is, sick persons with dyslipidemia die before inclusion in studies of elderly people, or that low total and LDL-C levels are part of an early, prodromal stage of MCI. In this stage, patients might show alterations in the energetic profile such as weight loss, reduced caloric intake and increased energy requirement,[11] and it is possible that low cholesterol and LDL-C levels reflect malnutrition in subjects with prodromal MCI. Also, lipid levels decrease with aging and may not have the same significance they have in middle age. Thus, it is possible that studies with shorter follow-up or higher baseline age of the participants might lack the ability to detect a potential harmful effect of elevated plasma lipids. We tried to eliminate these possibilities by repeating all analyses restricted to persons with longer follow-up but this did not change our results. Another potential explanation for our findings is that our study lacked statistical power to detect a small effect size. However, power calculation shows that, with a power of 80% and an alpha of 0.05 we were able to demonstrate relative risks for MCI of at least 1.30 for total cholesterol, 1.31 for HDL-C, 1.30 for triglycerides, and 1.32 for LDL-C. If there indeed is an association between plasma lipid levels and MCI it must be of relatively small magnitude. However, the HR and confidence intervals in our results were close to or lower than 1 and do not suggest that lack of power is an explanation for the lack of association.

The main limitation of this study was that we used only one measurement of lipid levels, which could have led to measurement error and an underestimation of the association between lipid levels and cognitive impairment. The main strength of our study is that it is a prospective cohort study designed for the diagnosis of cognitive impairment and dementia with standard criteria, and with complete clinical and neuropsychological evaluation at each interval that permitted the ascertainment of different types of incident MCI.

Our study does not support the hypothesis that lipids are important in cognition in the elderly, but is not in conflict with studies showing inverse associations in younger cohorts. Trials of lipid lowering treatment for the prevention of cognitive impairment are ongoing and will help clarify this question.

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2.4

Relation between Smoking and Risk of Dementia and Alzheimer's Disease

ABSTRACT

Background and Objective. Previous studies relating smoking with the risk of dementia have been inconsistent and limited in their validity by short follow-up times, large intervals between baseline and follow-up assessments, and unspecific determination of dementia diagnosis. We re-assessed after longer follow-up time in the large population-based cohort of the Rotterdam Study, whether smoking habits and pack-years of smoking are associated with the risk of dementia, Alzheimer's disease (AD), and vascular dementia (VaD). **Methods and Design.** Prospective population-based cohort study in 6,868 participants, 55 years or older and free of dementia at baseline. First, Cox proportional hazard models were used to relate smoking status at baseline with the risks of incident dementia, VaD and AD, using never smokers as the reference category in all analyses. Then Cox proportional hazard models were used to relate pack-years of smoking with the risks of incident dementia, VaD and AD. To explore the impact of the APOEε4 allele, sex, and age on the association between smoking status and dementia, we repeated all analyses stratifying, in separate models, by APOEε4 genotype, sex and median of age. **Results.** After a mean follow-up time of 7.1 years, current smoking at baseline was associated with an increased risk of dementia (HR 1.47, 95% CI 1.18-1.86) and AD (HR 1.56, 95% CI 1.21-2.02). This increase in disease risk was restricted to persons without the APOEε4 allele. There was no association between current smoking and risk of VaD, and there was no association between past smoking and risk of dementia, AD or VaD. **Conclusion.** Current smoking increases the risk of dementia. This effect is more pronounced in carriers without the APOEε4 allele than APOEε4 non-carriers.

INTRODUCTION

Smoking is a risk factor for many age-related chronic diseases such as cardiovascular disease and stroke.[1,2] Its association with dementia, one of the most common neurodegenerative diseases in the elderly and a major public health burden in western societies,[3] has been explored extensively. Several early case-control studies suggested that smoking might protect against Alzheimer's disease (AD).[4-13] Data from *in vitro* studies suggested that nicotine might be neuroprotective through anti-β-amyloid, anti-free radical, antiexcitotoxic, and amyloid precursor protein effects.[14-17] Results from autopsy studies have been inconsistent, reporting protective, adverse and no effects of smoking on AD pathology.[18-20]

In contrast to the findings by the early case-control studies, recent prospective cohort studies reported an increased risk[21-23] or unchanged risk[24-27] of AD in smokers compared with non-smokers, suggesting that biases inherent to case-control studies, such as selection and recall bias, might be an explanation for the early findings of a protective effect of smoking on AD. This explanation is further supported by studies reporting that a history of smoking was associated with an increased mortality among patients with dementia but not controls, suggesting that patients with dementia who have been smokers may be eliminated earlier from the population und thus might be underrepresented in cross-sectional samples.[27] Another explanation for the discrepant findings may be that some of the earlier studies that showed a protective effect of smoking included much younger cases. It is conceivable that the relation between smoking and Alzheimer's disease is age-dependent, for example, because of different genetic susceptibility.[23] Some support for this comes from the observation among early onset patients that the inverse association between smoking and Alzheimer's disease was limited to carriers of the APOE*4 allele.[28] In late-onset dementia smoking has been reported to increase the risk of dementia and AD.[23]

The validity of the recent prospective cohort studies, however, was also limited. Most of the studies had a short follow-up time,[21,22,24,25,27] leading to possible inclusion of persons with a pre-clinical stage of dementia in the study sample at baseline, and leading to a lack of ability to explore long-term effects of exposure on disease risk. The study by Doll et al.,[26] which had a longer follow-up time, relied on death certificate data to determine dementia,[26] implying the potential of misclassification of the outcome of interest and underreporting of mild cases of dementia. The Honolulu-Asia Aging Study (HAAS),[29] which reported an increasing risk of AD with increasing numbers of pack-years, had a long follow-up time with an interval of approximately 25 years between baseline and follow-up assessment, also leading to a potential to miss new cases.

The Rotterdam Study previously reported an increased risk of dementia and AD in current smokers without the APOEε4 allele after a mean follow-up time of 2.1 years.[23] The objective of

the present study was to re-assess the association between smoking and risk of dementia, AD and vascular dementia (VaD) in the same sample of the Rotterdam Study after longer study duration and with more incident dementia cases.

METHODS

Participants and Setting. The Rotterdam Study is a population-based prospective cohort study that was designed to investigate the incidence and causes of cardiovascular, neurological, endocrine, and ophthalmologic diseases in the elderly.[30] From 1990 to 1993, all 10,275 residents aged ≥ 55 years of Ommoord, a district of the city of Rotterdam, were invited to participate, and 7,983 (78%) men and women agreed. The Medical Ethics Committee of the Erasmus Medical Center approved the study, and written informed consent was obtained from all participants. During the baseline examination (1990-1993), a research assistant interviewed participants in their homes and obtained information on current and past health, medication, lifestyle, and risk factors for chronic diseases. In addition, participants visited the research center twice for baseline clinical examinations. Follow-up examinations took place in 1993-1994, 1997-1999, and 2002-2004. Through linkage with records of general practitioners, the entire cohort was continuously monitored for morbidity and mortality. This follow-up information was available for all participants until January 1, 2005.

From the 7,983 participants who underwent baseline examination, 7,528 were screened for dementia (94.3%). From these, 482 persons (6.4%) were excluded due to prevalent dementia, and 178 (2.4%) were excluded due to missing information on smoking history. The final analytic sample included in this study comprised 6,868 persons without dementia at baseline. Follow-up with respect to dementia was nearly complete (99.9%).

Diagnosis of Dementia and Alzheimer Disease. Diagnostic procedures for dementia and Alzheimer disease have previously been described in detail.[31] At baseline and all follow-up examinations, a three-stage protocol was used to screen all participants cognitively with the Mini-Mental State Examination (MMSE)[32] and the Geriatric Mental State schedule (GMS) organic level.[33] If subjects scored lower than 26 on the MMSE or higher than 0 on the GMS organic level, the Cambridge Examination of Mental Disorders in the Elderly (CAMDEX)[34] was administered. The CAMDEX also included an informant interview. Finally, participants in whom dementia was suspected were examined by a neurologist and neuropsychologist and, if possible, underwent magnetic resonance imaging of the brain. In addition, the total cohort was continuously monitored for incident dementia cases through computerized linkage between the study database and computerized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.[31] The diagnoses of dementia and

Alzheimer disease were based on *Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition* (*DSM-III-R*) criteria[35] and the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria,[36] respectively, and were made by a panel of a neurologist, neuropsychologist, and research physicians who reviewed all existing information.[31]

Assessment of smoking and other covariates. At baseline, trained investigators interviewed all participants at home, collecting information on socioeconomic status, current health status and medical history. In addition, clinical measures were obtained at the research center. Participants were asked about their current and past smoking habits. Those who smoked cigarettes were asked for their age at first smoking, the duration of time without smoking, and the average number of cigarettes smoked. Former smokers were asked about starting age, time without smoking, age when they stopped smoking, and average daily number of cigarettes smoked. Level of education was categorized into 3 groups: low (primary education only); intermediate (lower vocational or general education); and high (intermediate or higher vocational or general education, college, or university). Body mass index was calculated using the formula [weight (kg)/length (m^2)]. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. Diabetes mellitus was defined as a random or postload glucose level ≥ 11.1 mmol/L or a history of diabetes or the use of blood glucose-lowering medication. The amount of average daily alcohol consumption was assessed using a detailed questionnaire on food frequency.

Nonfasting blood samples were drawn and immediately frozen. Total cholesterol, high-density lipoprotein cholesterol, and glucose were measured within 2 weeks, as described previously.[37] Levels of serum C-reactive protein (CRP) were determined by the rate near infrared particle immunoassay method (Immage high-sensitivity CRP, Beckman Coulter).

Ultrasonography of both carotid arteries was performed. As an indicator of atherosclerosis of the carotid arteries, we used intima media thickness (IMT). Common carotid IMT was determined as the average of the maximum IMT of near- and far-wall measurements, and the average of left and right common carotid IMT was computed.[38] Apolipoprotein E (APOE) genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis.[39] After excluding persons with the APOEε2/ε4 genotype, we dichotomized APOE genotype into presence or absence of the apolipoproteinEε4 (APOEε4) allele.

Statistical Methods. First we evaluated the demographic and clinical characteristics of the study sample at baseline. Then we grouped individuals into never smokers, past smokers and current smokers at baseline. We calculated pack-year exposure by the average daily number of cigarettes divided by 20 and multiplied by the number of years smoked. We performed Cox proportional hazards analyses relating smoking status at baseline with the risks of incident dementia, VaD and AD, using never smokers as the reference category in all analyses. Then

we performed Cox proportional hazard models relating pack-years of smoking with the risks of incident dementia, VaD and AD. We initially adjusted all models for sex and age, then we adjusted for sex, age, APOEε4 genotype, education and alcohol intake in later analyses.

To explore the impact of the APOE ε4 genotype, sex, and age on the association between smoking status and risk of dementia, we repeated all analyses stratifying, in separate models, by APOEε4 genotype, sex and median of age. We finally repeated all analyses adding an interaction term to the model that contained variables for smoking status (current smoking yes/no, past smoking yes/no, and pack-years of smoking, respectively) and APOEε4 genotype. As described above, carriers of the APOEε2/ε4 genotype were excluded from all analyses since the APOEε2 allele seems to exert a protective effect on the risk of dementia and may counterbalance the effect of the APOEε4 allele.[40,41]

The time-to-event variable in all models was age at onset of dementia, death or end of follow-up, respectively. Persons who did not develop dementia, who died, or who were lost to follow-up owing to relocation before development of dementia were censored at the time of their last evaluation.

Data analysis was performed using SPSS version 13.0 software (SPSS Inc, Chicago, Ill) and STATA version 8 SE (StataCorp LP, College Station, TX).

RESULTS

There were 6,868 persons without dementia at baseline, with 49,949 person-years of follow-up (mean 7.3 person-years, SD 4.3 person-years). From these 6,868 individuals, 706 persons (10.3%) were diagnosed with dementia during follow-up. Out of those 706 persons diagnosed with dementia, 555 (78.6%) were diagnosed with AD, and 79 (11.2%) with VaD.

The baseline demographic and clinical characteristics of the study sample are shown in table 1.

In Cox proportional hazards analyses relating smoking status with the risk of incident dementia and AD, current smokers at baseline had a higher risk of dementia (HR 1.47, 95% CI 1.18-1.86) and AD (HR 1.56, 95% CI 1.21-2.02) than never smokers after adjusting for age and sex (table 2). These associations remained stable in models additionally adjusting for amount of alcohol intake and education, and models additionally adjusting for intake of antioxidants. When the analyses were restricted to VaD as the outcome, there was no association between smoking status and risk of VaD in any model. There was no association between past smoking and the risk of dementia, AD or VaD (table 2).

When the analyses were repeated stratifying by APOEε4 genotype, current smokers without an APOEε4 allele had a significantly increased risk of dementia (HR 1.66, 95% CI 1.14-2.42) and AD (HR 1.95, 95% CI 1.29-2.95), while there was no association in APOEε4 carriers (table 3).

Table 1. Baseline characteristics of the study sample in 6868 persons followed prospectively

Women, n (%)	4221 (59.9)
Age, mean (SD), year	69.5 (9.1)
Educational level	
Low	2599 (36.9)
Intermediate	1840 (26.1)
High	2386 (33.9)
Smoking, n (%)	
Never	2445 (35.6)
Past	2855 (41.6)
Current	1568 (22.6)
APOEε 4/- or 4/4 genotype, n (%)	1798 (25.5)
Alcohol intake (g/day), mean (SD)	10.4 (15.2)
Diabetes mellitus, n (%)	730 (10.4)
Hypertension, n (%)	4172 (59.2)
Body mass index (kg/m^2), mean (SD)	26.3 (3.9)
Intima media thickness (mm), mean (SD)	0.8 (0.2)

Table 2. Hazard ratios and 95% confidence intervals, relating smoking status and the risk of incident dementia, AD and VaD

	Incident Dementia		Incident AD		Incident VaD	
	No. (%)	HR (95% CI)	No. (%)	HR (95% CI)	No. (%)	HR (95% CI)
Model 1						
Never smokers	317 (13.0)	1.0 (reference)	267 (11.1)	1.0 (reference)	28 (1.3)	1.0 (reference)
Past smokers	262 (9.2)	1.15 (0.95-1.39)	192 (6.9)	1.17 (0.94-1.44)	36 (1.4)	1.18 (0.65-2.15)
Current smokers	127 (8.1)	1.47 (1.18-1.86)	96 (6.2)	1.56 (1.21-2.02)	15 (1.0)	1.37 (0.67-2.79)
Model 2						
Never smokers	317 (13.0)	1.0 (reference)	267 (11.1)	1.0 (reference)	28 (1.3)	1.0 (reference)
Past smokers	262 (9.2)	1.17 (0.92-1.48)	192 (6.9)	1.17 (0.90-1.52)	36 (1.4)	1.26 (0.59-2.68)
Current smokers	127 (8.1)	1.42 (1.07-1.89)	96 (6.2)	1.51 (1.10-2.08)	15 (1.0)	1.10 (0.43-2.84)

Model 1: adjusted for sex and age; Model 2: adjusted for age, sex, alcohol intake, and education

There was no association between smoking habit and risk of VaD in any strata of APOEε4 genotype, and there were no differences in the association between current or past smoking and risk of dementia, AD or VaD between strata of sex or median of age. When we repeated all analyses adding an interaction term to the model that contained variables for smoking status (current smoking yes/no and past smoking yes/no, respectively) and APOEε4 genotype, there was no interactive effect of current or past smoking with APOEε4 genotype on the risk of dementia, AD or VaD. When we compared the risk of dementia and AD among APOEε4 carriers who smoked with APOEε4 carriers who never smoked, using never smokers without APOEε4 genotype as the reference category, APOEε4 carriers who smoked had a higher risk of dementia (HR 2.78, 95% CI 2.18-3.55, p<0.0001) and AD (HR 3.08, 95% CI 2.36-4.02, p<0.0001) than

Table 3. Hazard ratios and 95% confidence intervals, relating smoking status and the risk of incident dementia, AD and VaD, stratified by APOEε4 genotype

	Incident Dementia		Incident AD		Incident VaD	
	No. (%)	HR (95% CI)	No. (%)	HR (95% CI)	No. (%)	HR (95% CI)
APOEε -/-						
Never smokers	174 (10.6)	1.0 (reference)	145 (9.0)	1.0 (reference)	20 (1.3)	1.0 (reference)
Past smokers	126 (6.7)	1.16 (0.83-1.62)	87 (4.7)	1.10 (0.76-1.60)	19 (1.1)	1.11 (0.40-3.08)
Current smokers	68 (6.5)	1.66 (1.14-2.42)	54 (5.2)	1.95 (1.29-2.95)	7 (0.7)	0.91 (0.25-3.30)
APOEε 4/- or 4/4						
Never smokers	105 (18.3)	1.0 (reference)	86 (15.5)	1.0 (reference)	7 (1.5)	1.0 (reference)
Past smokers	122 (15.5)	1.04 (0.73-1.49)	96 (12.6)	1.08 (0.73-1.60)	15 (2.2)	1.59 (0.49-5.12)
Current smokers	44 (11.0)	1.06 (0.67-1.69)	33 (8.5)	1.06 (0.62-1.79)	4 (1.1)	0.84 (0.14-4.93)

All models are adjusted for age, sex, alcohol intake and education

APOEε4 carriers who never smoked (incident dementia: HR 2.32, 95% CI 1.82-2.97, p<0.0001; incident AD: 2.38, 95% CI 1.82-3.11, p<0.0001)

In analyses relating the number of pack-years at baseline as a continuous variable with the risk of dementia in current smokers, increasing number of pack-years was associated with an increased risk of dementia (HR 1.01, 95% CI 1.001-1.008, p=0.06) and AD (HR 1.01, 95% CI 1.002-1.010, p=0.06) that was close to statistical significance. Exploring pack-years as a dichotomized variable, current smokers at baseline with exposure of more than 20 pack-years had a higher risk of dementia (HR 1.71, 95% CI 1.25-2.33) and AD (HR 1.82, 95% CI 1.26-2.57) than current smokers with exposure of less or equal to 20 pack-years (HR 1.52, 95% CI 1.15-2.11, and HR 1.60, 95% CI 1.15-2.26, respectively).

When these analyses were stratified by APOEε4 genotype, these associations became more pronounced in persons without APOEε4 genotype, while there was no association in APOEε4 carriers. There was no relation between amount of pack-years smoked and risk of VaD in current smokers, and there was no association between past smoking and risk of dementia, AD and VaD.

When we repeated all analyses adding an interaction term to the model that contained variables for pack-years of smoking and APOEε4 genotype, there was no interactive effect of number of smoking-pack years and APOEε4 genotype on the risk of dementia, AD or VaD.

DISCUSSION

We found an association between current smoking at baseline and an increased risk of dementia and AD that was restricted to persons without the APOEε4 allele. There was no association between current smoking and risk of VaD, and there was no association between past

smoking and risk of dementia, AD or VaD. There was no interactive effect of APOEε4 genotype and current or past smoking on the risk of dementia, AD or VaD.

A limitation of this study might be the ascertainment of smoking status. We relied on self-report by the participants. However, if misclassification of smoking has occurred, it is likely to be non-differential, resulting in an underestimation of the association between smoking and dementia. Given that we excluded subjects with dementia at baseline from the analyses, and given that the mean follow-up time in this study was relatively long (7.1 years), it seems unlikely that the report of smoking status at baseline was influenced by cognitive status. A common limitation associated with investigating effects of smoking in disorders with low incidence before old age is differential mortality. Smoking is related to higher mortality from various causes, and it is possible that some smokers would have demonstrated cognitive decline had they not died prior to inclusion in this cohort.

This study has important strengths. It was based on the population-based cohort of the Rotterdam Study with a large number of incident dementia cases, an average follow-up time of 7.1 years, a high response rate and virtually complete follow-up with respect to dementia.

Most of the previous longitudinal studies relating smoking habit with the risk of dementia were observational studies with a short follow-up time of approximately 2-3 years.[21,22,24,25,27] These studies implied the potential that some participants diagnosed with dementia during follow-up might have had slight cognitive impairment at baseline. This, in turn may have resulted in diagnosis of cases at follow-up which already have had biological disease at study entry, and in recall bias with misclassification of exposure status among cases.[42] Also, the short interval between baseline examination and follow-up in these studies limited their ability to assess the long-term effect of smoking on disease risk.

Interpretation of previous longitudinal studies with a longer follow-up period was also limited by methodological issues. Doll et al.[26] did not find an association between smoking habit and risk of AD in 34,439 male british doctors after 6 and 12 years of follow-up. This study relied on death certificates for the diagnosis of dementia and AD, which leaves room for missing cases with "undetected" dementia. Nondifferential misclassification of diagnosis (i.e. missing persons who died with unrecorded dementia, irrespective of smoking) could have driven the observed association towards the null.

The HAAS [29] reported an increasing risk of AD with increasing numbers of pack-years in 3,734 Japanese-American men after an interval of approximately 25 years between baseline examination in mid-life and follow-up assessment in late-life. Although this design nicely provides the ability to assess long-term effects of smoking on the risk of dementia, the findings also have to be interpreted with the caveat that, due to the long period between baseline and follow-up assessment, incident cases of dementia might have been missed. Also, restriction

of the study population to men of Japanese-American ancestry limits the generalizability of the results.

There are several pathways through with smoking might be associated with the risk of AD. It may increase the risk of dementia through cerebrovascular disease,[2] or could augment cholinergic metabolism by upregulation of cholinergic nicotinic receptors in the brain.[43] Cholinergic deficits, characterized by reduced levels of acetylcholine and nicotinic receptors, are found in AD.[44] However, nicotine increases acetylcholine release, elevates the number of nicotinic receptors, and improves attention and information processing.[45,46] These actions may be opposed by high oxidative stress caused by smoking, which is a putative mechanism in AD, through generation of free radicals and affecting inflammatory-immune systems, which activate phagocytes that generate further oxidative damage.[47-49] There is also evidence that smokers have a lower dietary intake of antioxidants compared with nonsmokers.[50]

In the present study with a mean follow-up time of 7.1 years, and an almost complete follow-up with respect to dementia, we found an association between current smoking at baseline and an increased risk of dementia and AD that was restricted to persons without the APOEε4 allele. These results are consistent with our previous observations of an increased risk of dementia and AD in persons without the APOEε4 allele after a mean follow-up time of 2.1 years,[23] and are in line with findings by Carmelli et al.,[51] reporting a lower cognitive function in smokers without the APOEε4 allele compared with non-smokers. They are also in agreement with observations by the Washington Heights Inwood Columbia Aging Project (WHICAP) reporting an increased risk of AD in current smokers without the APOEε4 allele in 1062 persons after a follow-up time of approximately 2 years,[22] and a faster cognitive decline in memory performance over 5 years in current smokers over the age of 75 years without the APOEε4 allele.[52]

There are several potential explanations for these findings. The presence of the APOE-ε4 allele increases the risk of AD.[53] Individuals with the APOE-ε4 genotype may have an increased risk of AD in such a way that other risk factors do not increase the risk further. Another potential explanation for the lack of an association of smoking to AD in APOEε-4 carriers is that smoking may be harmful through vascular mechanisms, but also partly beneficial in APOEε4 carriers. This hypothesis is supported by previous findings that persons with AD who are APOEε4 carriers have fewer nicotinic receptor binding sites and lower activity of choline acetyltransferase than non-carriers.[54] Smoking could counterbalance the APOEε4 associated impairment by facilitating the release of acetylcholine or increasing the density of nicotinic receptors. However, the facts that there was no interactive effect of (current) smoking and APOEε4 genotype on the risk of dementia or AD, and that APOEε4 carriers who smoked had - if any- a higher risk of dementia than APOEε4 carriers who never smoked, rather support the hypothesis that smok-

ing in fact increases the risk of dementia, but that this effect is less pronounced in persons who already are at increased risk by having APOEε4 genotype.

An alternative explanation for our findings may be that elderly smokers who are APOEε4 carriers are simply a selected group. The APOEε4 allele increases the risk of cardiovascular disease, and it is possible that mortality is disproportional high among these persons.[55] In the sample of the present study, however, mortality during follow-up was similar in carriers and non-carriers of the APOEε4 allele. This makes differential mortality an unlikely explanation for our findings.

We did not find an association between smoking and the risk of vascular dementia. This is in line with the findings by the WHICAP study observing a faster decline over time explicitly in memory but not executive or language performance in current smokers without the APOEε4 allele.[52] They are also consistent with observations by the HAAS reporting a relation between amount smoked and Alzheimer-type neuropathology,[29] as well as a relation between amount smoked and an increased risk of AD that was independent from inclusion or exclusion of persons with cerebrovascular disease.[29] An alternative explanation would be that elderly persons with VaD are at a disproportional higher risk of mortality.[55] In the sample of the present study, however, this was not the case, making differential mortality an unlikely explanation for the lacking association between current or past smoking and VaD.

In summary, this study confirms previous findings of studies with shorter follow-up of a relation between current smoking and an increased risk of dementia and AD in persons without the APOEε4 allele. The study suggests that –at least in elderly persons- this association is not based on a partly beneficial effect in APOEε4 carriers, but rather caused by a harmful effect of smoking on the risk of dementia that is less pronounced in persons who already are at increased risk by having an APOE-ε4 genotype.

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2.5

Effect of smoking and time on cognitive function in the elderly without dementia

ABSTRACT

Objective. To examine the association between smoking and changes in cognitive function over time in elderly persons without dementia. **Methods.** The results of neuropsychological tests grouped into domains of memory, abstract-visuospatial and language, from several intervals over a five-year-period in 791 elderly without dementia or cognitive impairment. Smoking history was categorized as never, current or past smokers and related to the slope of performance in each cognitive domain using generalized estimating equations. **Results.** Performance in all cognitive domains declined over time. Memory performance declined more rapidly among current smokers over age 75 years than in non-smokers similar in age, including those who never smoked or had quit smoking. The effect was stronger among those without an APOEε4 allele. There was no association between smoking and performance in any cognitive domain in persons under age 75 years, and there was no association between past smoking and performance on any of the three cognitive factors at any time interval in either age group. **Conclusion.** Current smokers over age 75 years perform more poorly on cognitive tests and appear to decline in memory more rapidly than their peers who do not smoke, especially if they lack the APOEε4 allele. Smoking does not affect cognitive performance in those persons under age 75 years.

INTRODUCTION

Cognitive decline is a major public health concern in aging societies. About 1 percent of people aged 65-69 years have dementia, and this proportion increases with age to approximately 60 percent for people over the age of 95.[1] There are inconclusive data relating smoking, a modifiable risk factor associated with many age-related diseases such as atherosclerosis or cerebrovascular disease,[2,3] to cognitive decline and dementia.[4-6] While case-control studies suggest that smoking lowers the risk of Alzheimer's Disease (AD),[6] prospective studies have shown an increased risk,[4,5,7] or no association with AD.[8-10] The effects of nicotine-induced increases in nicotinic acetylcholine receptors (nAChR) and protection against age-related nAChR decline are inconsistent because studies have also shown a reduction in nAChR in AD.[11]

Whether or not smoking affects cognitive function in elderly without dementia or cognitive impairment, remains unclear. Most of the evidence derives from retrospective or cross-sectional studies using only a single time-point for the analysis.[12,13] Longitudinal studies have provided only global neuropsychological assessments, did not have the ability to detect early stages of cognitive decline[14-16] or provided only short-follow-up periods.[8,9,16] The objective in this study was to determine whether or not smoking is associated with decline in memory and other cognitive functions in elderly persons without dementia or cognitive impairment without dementia (CIND) at baseline.

METHODS

Subjects and Setting. Participants were part of a longitudinal study of Medicare recipients 65 years or older residing in northern Manhattan (Washington Heights, Hamilton Heights, Inwood) that has been described elsewhere.[17] Each participant underwent an in-person interview of general health and function at the time of study entry followed by a standard assessment, including medical history, physical and neurological examination as well as a neuropsychological battery.[18] Baseline data were collected from 1992 through 1994. Follow-up data were collected during evaluations at sequential intervals of approximately 18 months, performed from 1994 to 1996, 1996 to 1997, and 1997 to 1999. In this elderly population, some participants did not complete follow up at all intervals due to refusal to participate further, relocation or death. About one half of participants were evaluated at the third follow-up visit. This study was approved by the institutional review board of the Columbia-Presbyterian Medical Center.

The participants selected for this study were without dementia or cognitive impairment, complete smoking information, and with at least 3 follow-up intervals.

Of the 2126 individuals who underwent clinical assessment at baseline, 346 (16.3%) individuals were excluded due to dementia or CIND at baseline. Information on smoking habit

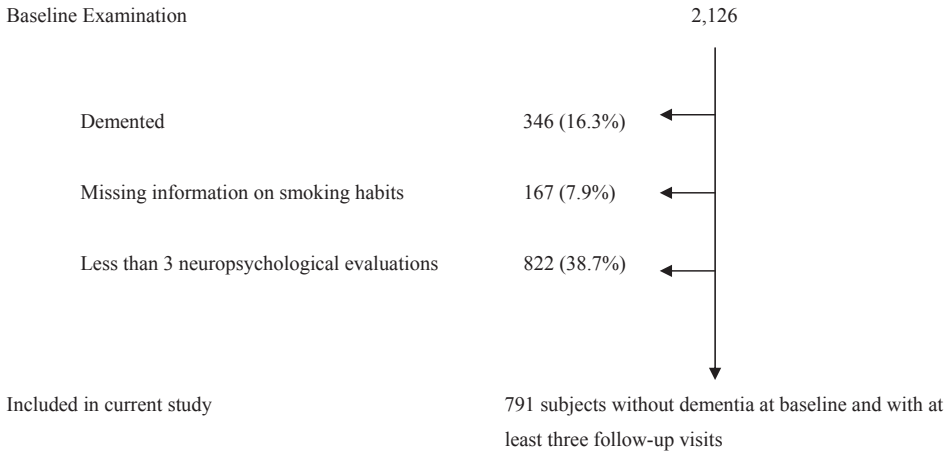

Figure 1. Description of sample size

was unavailable in 167 (7.9%) cases and 822 (38.7%) subjects had less than three follow-up visits with neuropsychological evaluation (Figure 1). The study focused on 791 individuals without dementia or cognitive impairment followed over a 5 year interval.

Clinical assessments. Data included medical, neurological, and neuropsychological evaluations.[18,19] All participants underwent a standardized neuropsychological test battery in either English or Spanish.[18] Orientation was evaluated using parts of the modified Mini-Mental State Examination.[20] Language was assessed using the Boston Naming Test,[21] the Controlled Word Association Test,[22] category naming, and the Complex Ideational Material and Phrase Repetition subtests from the Boston Diagnostic Aphasia Evaluation.[23] Abstract Reasoning was evaluated using WAIS-R Similarities subtest,[24] and the non-verbal Identities and Oddities subtest of the Mattis Dementia Rating Scale.[25] Visuospatial ability was examined using the Rosen Drawing Test,[26] and a matching version of the Benton Visual Retention Test.[27] Memory was evaluated using the multiple choice version of the Benton Visual Retention Test[27] and the seven subtests of the Selective Reminding Test:[28] total recall, long-term recall, long-term storage, continuous long-term storage, words recalled on last trial, delayed recall, and delayed recognition. This neuropsychological test battery has established norms for the same community.[29]

Definition of dementia and cognitive impairment. Results from the neurological, psychiatric and neuropsychological examinations were reviewed in a consensus conference comprised of physicians, neurologists, neuropsychologists and psychiatrists. Based on this review all participants were assigned to one of three categories: normal cognitive function, CIND, or dementia. A diagnosis of CIND required a) a memory complaint b) objective impairment in at least one cognitive domain based on the average of the scores on the neuropsychological measures within that domain and a 1.5 SD cutoff using normative corrections for age, years of

education, ethnicity and sex, c) essentially preserved activities of daily living, d) no evidence for dementia. Dementia was defined as the presence of abnormalities in several cognitive domains in neuropsychiatric testing accompanied by significant functional impairment (Clinical Dementia Rating (CDR) ≥ 1).

Smoking. A structured risk factor questionnaire, given in English and Spanish, was developed for the assessment of exposures to putative risk factors related to dementia.[30] A trigger question asked whether or not the individual ever smoked at least one cigarette per day for a period of one year or more. If the answer to the trigger question was no, the subject was classified as non-smoker and no further questions were asked. Participants who answered the question affirmatively were classified as current smokers when they were still smoking, or past smokers when they had quit smoking. Current and past smokers were additionally asked at what age they began smoking and how many cigarettes on average they had smoked or still smoked per day. Past smokers were also asked at what age they had stopped smoking.

APOE Genotyping. APOE genotypes were determined as described by Hixson and Vernier[31] with slight modification.[32] We classified persons as homozygeous or heterozygeous for the APOE ε4 allele or not having any ε4 allele.

Other covariates. Diabetes mellitus and hypertension were defined by self-report at baseline and at each follow-up interval or by the use of disease specific medications. Blood pressure measurements were also considered in the definition of hypertension. Body mass index (BMI) was calculated by the formula BMI = weight (Kg)/height $(m)^2$.

Statistical Methods. A factor analysis was performed using data from the entire cohort with the 15 neuropsychological measures using a principal component analysis with varimax rotation and Kaiser normalization.[33] This analysis resulted in three factors: 1) a memory factor, in which the seven subtests of the Selective Reminding Test were the main contributors; 2) a abstract/visuospatial factor, where visuospatial and tests of reasoning were the main contributors; and 3) a language factor, in which language measures from the Boston Naming Test,[21] Controlled Oral Word Association Test,[22] and the WAIS-R Similarities[24] were the main contributors. We calculated cognitive scores for each participant at each visit by adding the scores of the measures that contributed most to each factor (tests with correlations of 0.5 or higher). Each factor score was normally distributed.

GEE[34] were used to examine changes in each cognitive domain over time. The dependent variables were the factor scores, and the independent variables were current smoking, past smoking, time (included as a continuous variable), and the interaction of smoking and time. Gender, age, education, ethnic group, APOEε4 allele, hypertension and heart disease were included as covariates in subsequent analyses.

The GEE analysis yielded coefficient values that represent the associations between a factor score and variables included in the model. There were three main coefficients of interest in each model: one comparing the smoking groups at baseline, one relating the change in cognitive scores with time, and an interaction term for current or past smoking and time. A significant p value for the coefficient comparing smoking groups at baseline indicates a difference between two groups at baseline. A significant p value for the coefficient of time indicates a statistically significant change in a cognitive score over the total duration of follow-up. A significant p value for the interaction coefficient indicates a difference in the rate of change in a factor score depending on the smoking group; this is the main variable of interest for the interpretation of the analyses. All analyses were repeated after stratifying for median of age.

RESULTS

The mean age of the sample was 75.6 ± 5.4 years, 70.5% were women, 48.6% were Hispanic, 19.2% were White, and 31.6% were Black (Table 1). The mean of years of education was 8.7 ± 4.6, and 29.4% were homozygous or heterozygous for the APOE-ε4 allele. The mean BMI was 27.1 ± 5.1, and 16.9% of the subjects reported having diabetes, 56.8% hypertension and 14.6% heart disease. 48.9% were never smokers, 35.1% past smokers and 15.9% current smokers.

Men were more often current or past smokers than women (Table 2). Blacks were significantly less often never smokers but more often current smokers than Whites and Hispanics.

In the GEE analysis memory, abstract/visuospatial and language performance declined significantly over time. Increased age at baseline was related to lower scores in all three cognitive domains at each interval, while higher education and White ethnicity were associated with higher scores in all domains at each interval. Current or past smoking was not associated with more rapid cognitive decline in analyses for the whole sample (p for interaction of smoking and time = 0.2).

These analyses were repeated stratifying by median of age (75.6 years). Current smokers over 75 years showed significantly lower scores in abstract/visuospatial performance at baseline than never or past smokers (Table 3), and they showed a significant decline over the follow-up in memory ($p = 0.05$). Thus, memory performance declined at a faster rate among current smokers older than 75 years than in subjects of similar age who never smoked or quit smoking (Table 4). These associations remained significant after adjusting for age, gender, ethnic group, education, APOEε4 allele and potential vascular risk factors such as hypertension and heart disease. In participants without the APOEε4 allele being over 75 years smoking substantially increased the risk of cognitive and memory decline, while carriers of APOEε4 showed no relation between smoking and memory or abstract/visuospatial performance (Table 5).

Table 1. Demographic characteristics of the study population

	Healthy elderly (n=791)
Men	233 (29.5)
Women	558 (70.5)
Education, mean (SD), year	8.7 (4.6)
Age, mean (SD), year	75.6 (5.4)
Body mass index, mean (SD)	27.1 (5.1)
Ethnic group ‡	
White/Non-Hispanic	152 (19.2)
Black/Non-Hispanic	250 (31.6)
Hispanic	384 (48.6)
APOE genotype 4/4	13 (1.6)
APOE genotype 4/-	220 (27.8)
APOE genotype -/-	549 (69.4)
Smoking Habit	
Never smoker	387 (48.9)
Past smoker	278 (35.1)
Current smoker	126 (15.9)
No Diabetes	652 (82.4)
Diabetes, not treated	29 (3.7)
Diabetes, treated	104 (13.2)
No heart disease	670 (84.7)
Heart disease, not treated	22 (2.8)
Heart disease, treated	93 (11.8)
No hypertension	338 (42.7)
Hypertension, not treated	127 (16.6)
Hypertension, treated	318 (40.2)

Values are expressed as number (percentage) unless otherwise indicated. Some percentages are based on an incomplete sample due to small amounts of missing data. ‡ Classified by self-report using the format of the 1990 US census.[49]

Table 2. Comparison of smoking status by demographics in 791 subjects

	Never Smoking	Past Smoking	Current Smoking
Men	61 (26.2)	113 (48.5) *	59 (25.3) *
Women	326 (58.4) *	165 (29.6)	67 (12.0)
Ethnic group †			
White/Non-Hispanic	78 (51.3) *	59 (38.8)	15 (9.9)
Black/Non-Hispanic	104 (41.6)	86 (34.4)	60 (24.0) **
Hispanic	203 (52.9) *	133 (34.6)	48 (12.5)

Values are expressed as number (SD) unless otherwise indicated. Some percentages are based on an incomplete sample due to small amounts of missing data. * Significant at a 0.05 level versus lowest value within smoking group, based on χ^2 test for categorical data. ** Significant at a 0.05 level versus all lower values within smoking group, based on χ^2 test for categorical data. † Classified by self-report using the format of the 1990 US census.[49]

Table 3. Impact of current smoking and follow-up time on abstract/visuospatial performance in elderly persons stratified by age group

	Model 1		Model 2	
Variable	**Estimated ß (SE)**	**p-value**	**Estimated ß (SE)**	**p-value**
Persons ≤ 75 years old				
Time	-0.7 (0.2)	0.001*	-0.7 (0.2)	0.001*
Current Smoking	1.2 (2.7)	0.7	0.2 (2.9)	0.9
Time*current smoking	0.1 (0.5)	0.9	0.1 (0.5)	0.9
Persons > 75 years old				
Time	-0.9 (0.2)	0.002*	-1.0 (0.2)	0.002*
Current Smoking	-7.4 (2.3)	0.001*	-7.9 (2.4)	0.001*
Time*current smoking	-0.5 (0.6)	0.4	-0.4 (0.5)	0.5

Model 1 is adjusted for age and gender, Model 2 is adjusted for age, gender, education, ethnic group and APOEε4, hypertension, heart disease and diabetes

Table 4. Relationship of current smoking and time of follow-up to memory performance in elderly persons over 5 years of follow-up stratified by age group

	Model 1		Model 2	
Variable	**Estimated ß (SE)**	**p-value**	**Estimated ß (SE)**	**p-value**
Persons ≤ 75 years old				
Time	-5.9 (0.6)	0.001*	-5.8 (0.6)	0.001*
Current Smoking	-1.0 (5.8)	0.8	-3.6 (5.8)	0.9
Time*current smoking	-1.2 (1.6)	0.4	-1.1 (1.6)	0.5
Persons > 75 years old				
Time	-7.7 (0.6)	0.002*	-7.9 (0.7)	0.002*
Current Smoking	-1.8 (1.2)	0.1	-3.9 (6.6)	0.5
Time*current smoking	-0.7 (0.3)	0.05*	-4.0 (1.8)	0.02*

Model 1 is adjusted for age and gender, Model 2 is adjusted for age, gender, education, ethnic group and APOEε4, hypertension, heart disease and diabetes

There was no association between smoking and decline in language or abstract-visuospatial test (Tables 3 and 6). Scores of both factors were normally distributed at each time interval indicating that the lack of a total current smoking*time interaction was not the result of a ceiling or floor effect.

DISCUSSION

In this study the performance in memory, abstract-visuospatial and language domains over time declined in individuals free of dementia or cognitive impairment at baseline, and increased age was associated with lower scores in all cognitive domains. Current smoking was

Table 5. Relationship of current smoking and time of follow-up to memory and abstract/visuospatial performance by APOEε4 genotype

	-/- APOEε4 genotype		-/4 or 4/4 APOEε4 genotype	
Variable	**Estimated ß (SE)**	**p-value**	**Estimated ß (SE)**	**p-value**
Memory Performance				
Persons ≤ 75 years old				
Time	-5.5 (0.7)	0.001*	-6.9 (1.2)	0.002*
Current Smoking	-5.7 (6.6)	0.4	8.9 (11.2)	0.4
Time*current smoking	-1.3 (1.9)	0.5	-0.1 (2.7)	0.9
Persons > 75 years old				
Time	-7.1 (0.7)	0.001*	-9.7 (1.2)	0.002*
Current Smoking	-4.8 (7.7)	0.5	0.4 (10.7)	0.9
Time*current smoking	-5.5 (2.3)	0.016*	-0.9 (2.8)	0.7
Abstract/visuospatial Performance				
Persons ≤ 75 years old				
Time	-0.7 (0.2)	0.003*	-0.8 (0.4)	0.08
Current Smoking	-1.8 (3.4)	0.6	5.1 (5.2)	0.3
Time*current smoking	0.4 (0.6)	0.5	-0.6 (1.0)	0.5
Persons > 75 years old				
Time	-0.9 (0.3)	0.001*	-1.2 (0.4)	0.006*
Current Smoking	-8.9 (3.1)	0.005*	-4.7 (4.0)	0.3
Time*current smoking	-0.3 (0.7)	0.7	-0.4 (1.2)	0.7

All models adjusted for age, gender, education, ethnic group, hypertension, heart disease and diabetes

associated with faster cognitive decline only in memory among subjects older than 75 years without the APOE-ε4 allele.

Past smoking was not associated with poor performance in any cognitive domain at any specific time interval, or decline in any domain over time.

The mechanisms by which smoking affects cognitive performance remain unclear. It has been proposed that smoking may increase the risk of dementia through cerebrovascular disease,[35] or that it augments cholinergic metabolism by upregulation of cholinergic nicotinic receptors in the brain.[36] Cholinergic deficits, characterized by reduced levels of acetylcholine and nicotinic receptors, are found in AD.[37] However, nicotine increases acetylcholine release, elevates the number of nicotinic receptors, and improves attention and information processing.[38] These actions may be opposed by high oxidative stress caused by smoking, which is a putative mechanism in AD,[39,40] through generation of free radicals and affecting inflamma-

Table 6. Relationship of current smoking and time of follow-up to language performance in healthy elderly over 5 years of follow-up stratified by age group

	Model 1		Model 2	
Variable	**Estimated ß (SE)**	**p-value**	**Estimated ß (SE)**	**p-value**
Persons ≤ 75 years old				
Time	-0.2 (0.1)	0.002*	-0.2 (0.1)	0.001*
Current Smoking	0.7 (0.4)	0.1	0.6 (0.4)	0.2
Time*current smoking	-0.1 (0.1)	0.4	-0.1 (0.1)	0.5
Persons > 75 years old				
Time	-0.3 (0.1)	0.003*	-0.3 (0.1)	0.004*
Current Smoking	-0.5 (0.6)	0.5	-0.7 (0.7)	0.3
Time*current smoking	-0.1 (0.2)	0.5	-0.1 (0.2)	0.5

Model 1 is adjusted for age and gender, Model 2 is adjusted for age, gender, education, ethnic group and APOEε4, hypertension, heart disease and diabetes

tory-immune systems, which activate phagocytes that generate further oxidative damage.[41] There is also evidence that smokers have a lower dietary intake of antioxidants compared with nonsmokers.[42]

Studies examining the role of smoking in cognitive function reported inconsistent results. Several case-control studies suggested that smoking might be related to a lower risk of AD,[6] but prospective studies reported an increased risk of AD[4,5,7] or no association.[8-10]

Our results are consistent with studies showing an increased risk of AD in current smokers. The main cognitive domain affected in AD is memory[43,44] and it seems reasonable to postulate that if smoking is related to a higher risk of AD, it must be related to decline in memory.

We found that the association between current smoking and AD was restricted to persons older than 75 years of age. The risk of AD increases with age,[43] and our finding may indicate that smoking increases the risk of memory decline in those who are more likely to develop memory decline. We also found that the association between current smoking and faster cognitive decline was confined to subjects without the APOE-ε4 allele. This is in agreement with two previous studies reporting an increased risk of AD in participants without the APOEε4 allele. The presence of the APOE-ε4 allele increases the risk of AD.[45] Older individuals with the APOE-ε4 may have an increased risk of memory decline[46] in a such a way that other risk factors may not increase the risk further. Another potential explanation for the lack of association of smoking to memory decline in APOEε-4 carriers is that smoking may be harmful through vascular mechanisms, but also partly beneficial in APOEε4 carriers. This hypothesis

is supported by previous findings that persons with AD who are APOEε4 carriers have fewer nicotinic receptor binding sites and lower activity of choline acetyltransferase than non-carriers.[47] Smoking could counterbalance the APOEε4 associated impairment by facilitating the release of acetylcholine or increasing the density of nicotine receptors.

There are several potential alternative explanations for our findings. One is chance, particularly in the context of multiple comparisons. However, our findings were not unexpected, are consistent with our previous findings relating current smoking to a higher risk of AD,[5] and consistent with other studies as described in the previous paragraph; these facts make chance due to multiple comparisons an unlikely explanation for our findings.[48] Another potential explanation is bias. For example, that only subjects with preclinical AD reported smoking while subjects that would not develop AD did not. This type of reporting bias seems unlikely and we excluded cases of incipient dementia or cognitive impairment that could have influenced our results. Another potential explanation is confounding. For example, if lower education is related to current smoking, and persons with lower education are more likely to be diagnosed with AD, then it is possible that a relation between smoking and cognitive decline could be due to confounding by socioeconomic factors. We adjusted for years of education and ethnicity as markers of socioeconomic status to account for this possibility. Finally, another explanation is genetic confounding. It may be that smoking propensity is associated with a gene or combination of genes (but not APOE) which in turn is associated with the risk of AD. Therefore, it is possible that smoking is related to other behaviors related to poor health or genetic factors, that in turn may increase the risk of AD, that we could not adjust for, and we cannot eliminate the possibility of lack of control for unknown confounders as a potential explanation for our findings.

This study has several strengths. We had a comprehensive and sensitive neuropsychological battery validated for use in the communities of northern Manhattan.[18] We also excluded from our analyses persons with dementia and cognitive impairment without dementia at baseline that may have biased the analyses, and had several evaluation time points that allowed prospective analyses.

The main limitation of this study is the ascertainment of smoking status. We relied on self-report by participants, and did not have information on quantity or duration of smoking. Assuming random misclassification of smoking, this would have resulted in the underestimation of the association between smoking and cognitive impairment. Given that we excluded subjects with dementia and with cognitive impairment without dementia at baseline from the analyses, it seems unlikely that the report of smoking status was influenced by cognitive status.

It is important to point out that this study was conducted in an elderly multiethnic community in an urban setting with a high prevalence of risk factors for morbidity and mortality,

such as diabetes and hypertension. Persons who dropped out of the study before completing at least three follow-up visits were at baseline older, less educated and had a higher prevalence of vascular risk factors than those who remained in the study. Also, smoking is related to higher mortality from various causes, and it is possible that many smokers would have demonstrated cognitive decline had they not died prior to inclusion in this cohort. Thus, there are important biases related to the sample of this study that should be taken into account in the interpretation and generalization of these findings.

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2.6

Hypertension and risk of mild cognitive impairment

ABSTRACT

Background and Objective. There are conflicting data relating hypertension to the risk of Alzheimer's disease (AD). We sought to explore whether hypertension is associated with the risk of mild cognitive impairment (MCI), a transitional stage to AD. **Design and Setting.** Prospective community-based cohort study conducted in northern Manhattan. **Methods.** Multivariate proportional hazards regression analyses, relating hypertension to incident all-cause MCI, amnestic MCI, and non-amnestic MCI in 918 persons without prevalent MCI at baseline followed for a mean of 4.7 years. **Results.** There were 334 cases of incident MCI, 160 cases of amnestic MCI and 174 cases of non-amnestic MCI during 4337 person years of follow-up. Hypertension was associated with an increased risk of all-cause MCI (HR 1.4, 95% CI 1.06-1.77, p=0.02) and non-amnestic MCI (HR 1.7, 95% CI 1.13-2.42, p=0.009) after adjusting for age and gender. Both associations were slightly attenuated in models additionally adjusting for stroke and other vascular risk factors. There was no association between hypertension and the risk of amnestic MCI (HR 1.1, 95% CI 0.79-1.63, p=0.49). There was no effect modification of the association between hypertension and MCI by APOEε4 genotype or use of antihypertensive medication. **Conclusion.** A history of hypertension is related to a higher risk of all-cause and non-amnestic MCI, but not amnestic MCI. These findings are consistent with the hypothesis that hypertension is primarily related to non-AD forms of cognitive impairment, which are increasingly recognized and of public health importance.

INTRODUCTION

Mild cognitive impairment (MCI) has attracted increasing interest over the past years, particularly as a means of identifying early stages of Alzheimer's Disease (AD) as a target for treatment and prevention. Studies using the criteria by Petersen et al. for diagnosing MCI in clinical and epidemiological settings,[1,2] report an incidence rate of 9.9/1,000 person-years for MCI among nondemented elderly,[3] and an annual conversion rate of 10% to 12% to AD in subjects with MCI, particularly amnestic MCI, in contrast to a conversion rate of 1% to 2% in the normal elderly population.[4]

There are inconclusive data relating hypertension, a modifiable vascular risk factor, to cognitive impairment and dementia. While most longitudinal studies reported an increased blood pressure before the onset of AD or vascular dementia (VaD),[5,6] most cross-sectional studies[7,8] or studies with shorter follow up[9] observed associations between low blood pressure and dementia, or no association between hypertension and cognitive impairment. We previously reported relations between hypertension and VaD but not AD. There are also conflicting data on the effect of antihypertensive treatment on cognition.[10,11]

The mechanisms underlying the associations between blood pressure and cognitive impairment or dementia remain unclear. High blood pressure levels may lead to white matter hyperintensities (WMH) on MRI or lacunar brain infarcts, which in turn may lead to cognitive impairment or dementia.[12,13] More direct links between blood pressure and AD are suggested by autopsy studies reporting an increased frequency of neurofibrillary tangles and brain atrophy in hypertensive persons.[14,15]

Our objective in the present longitudinal study was to determine whether or not hypertension is associated with the risk of incident MCI.

METHODS

Subjects and Setting. Participants were enrolled in a longitudinal cohort study by a random sampling of Medicare recipients 65 years or older residing in northern Manhattan (Washington Heights, Hamilton Heights, Inwood). The sampling procedures have been described elsewhere.[16] Each participant underwent an in-person interview of general health and function at the time of study entry followed by a standard assessment, including medical history, physical and neurological examination as well as a neuropsychological battery.[17] Baseline data were collected from 1992 through 1994. Follow-up data were collected during evaluations at sequential intervals of approximately 18 months, performed from 1994 to 1996, 1996 to 1997, and 1997 to 1999. In this elderly population, some participants did not complete follow up at

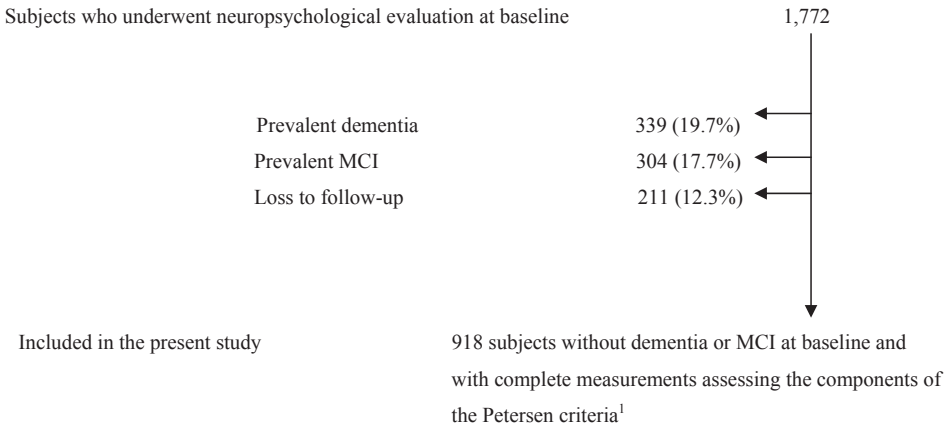

Figure 1. Description of sample size

all intervals due to refusal, relocation or death. About one half of participants were evaluated at the third follow-up visit. This study was approved by the institutional review board of the Columbia-Presbyterian Medical Center.

The sample for this study comprised those participants who were without MCI or dementia at baseline, who had at least one follow-up interval, and who had complete information to ascertain MCI following the Petersen criteria.[1,4] Of the 1,772 participants in whom a full neuropsychological exam was attempted, 339 (19.7%) were excluded due to prevalent dementia, 304 (17.7%) were excluded due to prevalent MCI, and 211 (12.3%) were excluded due to loss to follow-up (Figure 1). Thus, the final analytic sample included 918 individuals.

Compared to the original 1,772 participants, the final sample without prevalent MCI and dementia and with prospective data was younger 76.3 ± 6.1vs. 77.3 ± 6.8 years; $p < 0.0001$), and had a similar distribution of women (69.4 vs. 69.4%), African-Americans (33.6 vs. 32.6.3%), a lower proportion of Hispanics (43.9 vs. 47.0%; $p < 0.0001$), a higher proportion of Non-Hispanic Whites (22.6 vs. 20.4%; $p = 0.008$).

Clinical assessments. Data were available from medical, neurological, and neuropsychological evaluations.[17,18] All participants underwent a standardized neuropsychological test battery that examined multiple domains in either English or Spanish.[17] Orientation was evaluated using parts of the modified Mini-Mental State Examination.[19] Language was assessed using the Boston Naming Test,[20] the Controlled Word Association Test,[21] category naming, and the Complex Ideational Material and Phrase Repetition subtests from the Boston Diagnostic Aphasia Evaluation.[22] Abstract Reasoning was evaluated using WAIS-R Similarities subtest,[23] and the non-verbal Identities and Oddities subtest of the Mattis Dementia Rating Scale.[24] Visuospatial ability was examined using the Rosen Drawing Test,[25] and a matching version of the Benton Visual Retention Test.[26] Memory was evaluated using the multiple choice version

of the Benton Visual Retention Test[26] and the seven subtests of the Selective Reminding Test:[27] total recall, long-term recall, long-term storage, continuous long-term storage, words recalled on last trial, delayed recall, and delayed recognition. This neuropsychological test battery has established norms for the same community.[28]

Diagnosis of Dementia. Diagnosis of dementia and assignment of specific cause was made by consensus of neurologists, psychiatrists, and neuropsychologists based on baseline and follow-up information. The diagnosis of dementia was based on DSM-IV criteria[29] and required evidence of cognitive deficits on the neuropsychological test battery as well as evidence of impairment in social or occupational function (Clinical Dementia Rating of 1 or more).[30] Diagnosis of AD was based on the NINCDS-ADRDA criteria.[31]

Definition of MCI. MCI criteria were retrospectively applied among nondemented individuals after the consensus conference. Consistent with standard criteria[1,4] for all subtypes of MCI, those considered for MCI were required to have: 1) a memory complaint 2) objective impairment in at least one cognitive domain based on the average of the scores on the neuropsychological measures within that domain and a 1.5 SD cutoff using normative corrections for age, years of education, ethnicity, and sex, 3) essentially preserved activities of daily living (defined above), and 4) no diagnosis of dementia at the consensus conference.

In order to cast the widest net to determine the prevalence of MCI and to determine which individuals were more likely to progress to dementia, the original Petersen criteria,[1] which focus on memory impairment, were expanded to include mutually exclusive subtypes based on cognitive features. The first subtype, MCI-Amnestic (MCI-A), corresponds most closely to the original definition used by Petersen and colleagues. Memory impairment was defined as a score < 1.5 SD below demographically corrected mean on an average composite measure comprising the following learning and memory measures: 1) total recall from the SRT 2) delayed free recall from the SRT, and 3) recognition from the BVRT. Performance on composite scores from all other cognitive domains (i.e., executive, language, and visuospatial) was required to be within normal limits (score must be greater than or equal to 1.5 SD below the demographically corrected mean). Other MCI subtypes were classified that allowed for impairment in a single non-memory domain if performance on composite scores from all other cognitive domains was within normal limits. MCI-Executive Function (MCI-E) was assigned if impairment was demonstrated on an average composite measure comprising the following measures: 1) Letter Fluency; 2) Category Fluency, and 3) the WAIS-R Similarities subtest. MCI-Language (MCI-L) was defined as isolated impairment on an average composite measure comprising: 1) Boston Naming Test; 2) BDAE Repetition, and the 3) BDAE Comprehension test. MCI-Visuospatial (MCI-V) was assigned if impairment was demonstrated on an average composite score comprising: 1) Rosen Drawing and 2) BVRT matching. Finally, we allowed for impairment in multiple cognitive domains in the absence of dementia. MCI-Mul-

tiple Cognitive Domains with memory impairment (MCI-MCDM) was diagnosed if there was objective impairment on the memory domain composite score and if there was impairment on at least one other cognitive domain. MCI-Multiple Cognitive Domains without memory impairment (MCI-MCDN) was assigned if there was impairment in two or more of the three non-memory domains, and if the memory domain composite score was within normal limits. Again, classification into the six subtypes was mutually exclusive. We used three outcomes for these analyses: 1) all-cause MCI; 2) amnestic MCI, which included MCI-A and MCI-MCDM; and 3) non-amnestic MCI. The rationale for this classification is that MCI-A and MCI-MCDM equally predict the development of AD, and MCI-MCDM is thought to be a more advanced form of MCI-A involving other cognitive domains.

Definition of hypertension and other covariates. At baseline, all participants were asked whether or not they had a history of hypertension any time during their life. If affirmative, they were asked whether or not they were under treatment and the specific type of treatment. Stroke was defined according to the WHO criteria.[32] Blood pressure was also recorded at each visit using the Dinamap Pro 100 (Critikon Co., Tampa, FL). The blood pressure cuff was placed on the right arm while the individual was seated, and a recording was obtained every 3 minutes over 9 minutes. The third measurement was recorded in the database. Values above 140 mm Hg (systolic) and 90 mm Hg (diastolic) were used as criteria for hypertension.

The presence of stroke was ascertained from an interview with participants and their informants. Persons with stroke were confirmed through their medical records, 85% of which included results of brain imaging. The remainder was confirmed by direct examination. Diabetes mellitus was defined as a history at any time during life. At baseline, all participants were asked whether or not they had a history of diabetes. If affirmed, they were asked whether or not they were under treatment and the specific type of medication. Heart disease was defined as a history of atrial fibrillation and other arrythmias, myocardial infarction, congestive heart failure or angina pectoris at any time during life.

APOE Genotyping. APOE genotypes were determined as described by Hixson and Vernier with slight modification.[33] We classified persons as homozygeous or heterozygeous for the APOEε4 allele or not having any ε4 allele.

Statistical Methods. Information on demographic characteristics and other potentially relevant factors were compared among individuals with and without a history of hypertension. χ^2 tests were used for categorical data and analysis of variance for continuous variables. Multivariate Cox proportional hazard models were used to estimate the association of hypertension to incident all-cause MCI, amnestic MCI and non-amnestic MCI. The time-to-event variable was age at onset of MCI. Among individuals who did not develop MCI, those who developed dementia were censored at the time of dementia diagnosis, and those who did

not develop dementia, who died, or who were lost to follow-up owing to relocation before development of MCI were censored at the time of their last evaluation. Information on covariates was obtained at baseline. We initially adjusted for sex and age, then we adjusted for sex, age, race, education and APOEε4 genotype in a second model. In a third model we adjusted for sex, age, race, education, APOEε4 genotype, stroke, diabetes mellitus, heart disease, and plasma low-density lipoprotein (LDL)-Cholesterol level. The additional covariates in the third model are theoretically in the pathways linking hypertension and MCI. Thus, any attenuation of hazard ratios observed in this model should be interpreted as evidence of mediation, and not of confounding. To explore the association between blood pressure levels and risk of MCI, we finally repeated all analyses using the continuous measures of blood pressure as the independent variable. All data analysis was performed using SPSS version 13.0 software (SPSS Inc, Chicago, Ill).

RESULTS

There were 334 cases of incident MCI, 160 cases of amnestic MCI and 174 cases of non-amnestic MCI during 4337 person years of follow-up (incidence densities = 7.7, 3.7 and 4.0 cases, respectively, per 100 person-years of observation). The mean age of the sample was 76.3 ± 6.1 years, and 69.4% were women, 22.6% were white, 33.6% black and 43.9% were hispanic. The

Table 1. Comparison of characteristics among persons with and without hypertension in 918 subjects followed prospectively

	No hypertension (n=292)	Hypertension (n=626)
Women, n (%)	178 (61.0)	461 (73.6)*
Age, mean (SD), year	76.9 (6.6)	75.6 (5.7)
Education, mean (SD), year	9.8 (4.5)	8.4 (4.5)*
Ethnic group, n (%) †		
White/Non-Hispanic	84 (28.8)	116 (18.5)
Black/Non-Hispanic	101 (34.6)	207 (33.1)
Hispanic	105 (36.0)	298 (47.6)
APOE genotype 4/- or 4/4, n (%)	76 (27.9)	264 (26.5)
Stroke, n (%)	24 (8.2)	114 (18.2)*
Diabetes, n (%)	35 (12.0)	184 (29.4)*
Heart disease, n (%)	55 (18.8)	256 (40.9)*
Current Smoking, n (%)	33 (11.3)	62 (9.9)
LDL (mg/dl), mean (SD)	121.1 (36.3)	120.1 (36.9)
MCI, n (%)	76 (26.0)	251 (41.2)*

Some percentages are based on an incomplete sample due to small amounts of missing data. † Classified by self-report using the format of the 1990 US census.[46] * significant at a 0.05 level vs. group without hypertension. MCI = mild cognitive impairment. LDL = low-density lipoprotein (LDL) Cholesterol.

Table 2. Hazard ratios and 95% confidence intervals, relating hypertension and the risk of incident MCI

MCI subtype	No. (%) of Incident MCI	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 HR (95% CI)
All-cause MCI				
No hypertension	76 (26.0)	1.0	1.0	1.0
Hypertension	258 (41.2)	1.4 (1.06-1.77)*	1.3 (1.02-1.73)*	1.2 (0.81-1.69)
Amnestic MCI				
No hypertension	42 (14.4)	1.0	1.0	1.0
Hypertension	118 (18.8)	1.1 (0.79-1.63)	1.1 (0.80-1.67)	0.9 (0.54-1.47)
Non-amnestic MCI				
No hypertension	34 (11.6)	1.0	1.0	1.0
Hypertension	140 (22.4)	1.7 (1.13-2.42)*	1.6 (1.06-2.29)*	1.6 (0.93-2.85)

Cox proportional hazards model, with age-at-onset as time variable, as described in the text. Some percentages are based on an incomplete sample due to small amounts of missing data. HR=hazard ratio, 95% CI= 95 percent confidence interval. Model 1: adjusted for gender and age Model 2: adjusted for age, gender, education, race and APOE. Model 3: adjusted for gender, age, race, education, APOE, stroke, diabetes, heart disease, current smoking and LDL-cholesterol

mean of years of education was 8.7± 4.6, and 62.8% had hypertension, 21.3% diabetes, and 30.4% heart disease. 25.0% of the sample were homo- or heterozygeous for the APOEε4 allele, and use of antihypertensive medication was reported by 394 subjects (42.9%). Persons with hypertension were more often women, less educated, and had more often a history of stroke, diabetes or heart disease than persons without hypertension (table 1).

Risk of incident MCI. The mean age at onset of MCI was 80.7 ± 5.9 years. In multivariate analyses hypertension was associated with an increased risk of all-cause MCI (HR 1.4, 95% CI 1.06-1.77, p=0.02) and non-amnestic MCI (HR 1.7, 95% CI 1.13-2.42, p=0.009) after adjusting for age and gender (table 2). These associations remained stable in models additionally adjusting for education, race and APOEε4 genotype, and were slightly attenuated in models additionally adjusting for stroke and other vascular risk factors such as diabetes, LDL-Cholesterol, smoking or heart disease. The results did not change after adjusting for blood pressure measurements or use of antihypertensive medication. There was no relation between hypertension and the risk of amnestic MCI (HR 1.1, 95% CI 0.79-1.63, p=0.49) in either model. There was no effect modification of the association between hypertension and MCI by APOEε4 genotype. Using blood pressure measurements instead of diagnosis of hypertension as the independent variable, or restricting the analyses to persons with longer follow-up time (observation time ≥ the median follow-up time of 3.9 years) did not change the observed associations.

DISCUSSION

In this longitudinal analysis of 918 persons, hypertension was associated with an increased risk of all-cause MCI that was mostly driven by an association with an increased risk of non-amnestic MCI after adjusting for age and gender. There was no relation between hypertension history and the risk of incident amnestic MCI, an early stage of AD, and there was no effect modification of the association between hypertension and any MCI subtype by APOEε4 genotype or use of antihypertensive medication.

The mechanisms by which blood pressure affects the risk of cognitive impairment or dementia remain unclear. It has been proposed that hypertension may cause cognitive impairment through cerebrovascular disease. Hypertension is a risk factor for subcortical white matter lesions (WMLs) found commonly in AD.[34] Hypertension may also contribute to a blood-brain barrier dysfunction, which has been suggested to be involved in the aetiology of AD.[34] Other possible explanations for the association are shared risk factors, such as the formation of free oxygen radicals.[34,35]

In our study hypertension was associated with a higher risk of all-cause MCI and non-amnestic MCI. MCI has been described as an intermediate stage between normal cognition and dementia.[1,36] Non-amnestic MCI, as defined in our study, is likely to be related in particular to cerebrovascular disease and vascular cognitive impairment (VCI). Since hypertension is associated with a higher risk of cerebrovascular disease and vascular dementia,[37,38] it seems reasonable that it must be related with the risk of non-amnestic MCI. Also, the relation of hypertension to non-amnestic MCI remained stable after adjusting for education, race and APOEε4 genotype and was attenuated after adjustment for stroke and vascular risk factors, indirectly suggesting that cerebrovascular disease may be mediating the relation between hypertension and non-AD forms of MCI. Our results support the notion that hypertension is mainly related to an increased risk of non-amnestic forms of cognitive impairment,[39] such as frontal-executive cognitive impairment.

There was no relation between hypertension and the risk of incident amnestic MCI. Episodic memory deficits have been found to be a strong predictor of conversion to dementia, in particular AD.[40] Consequently, the term amnestic MCI represents a subgroup with a high probability of conversion to dementia caused by AD.[40] The association between hypertension and AD is unclear. A 15-year longitudinal study reported increased blood pressure 10-15 years before the onset of both AD and vascular dementia.[41] Others found it to be lower in old individuals with AD,[7] or did not find an association between hypertension and cognitive impairment.[42]

In the interpretation of these findings it is of major importance to keep in mind that MCI is likely to be a clinically and pathologically heterogeneous syndrome, and that definitions of MCI and MCI subtypes are not established diagnostic entities. The frequency of dementia in a group of individuals with cognitive impairment is the result of both the definition of the disorder and the underlying pathophysiology. Thus, it is possible that different definition of MCI or MCI subtypes would have led to different results. Our study does not exclude the possibility that hypertension is associated with a type of MCI that is related to the Alzheimer component of dementia.

There are alternative explanations for our observations. One is that hypertension is part of a pre-clinical syndrome of non-amnestic MCI, or that persons with pre-clinical non-amnestic MCI reported hypertension while subjects that would not develop MCI did not; we tried to eliminate these possibilities by excluding persons with baseline MCI from the analyses, and by repeating the analyses restricted to persons with longer follow-up time. Another potential explanation for our findings is chance due to multiple comparisons. However, the results are in line with the *a priori* hypothesis of an association between hypertension and non-amnestic MCI, and are mechanistically plausible. These facts make chance due to multiple comparisons an unlikely explanation for our findings.[43] Another potential explanation is confounding. For example, if lower education is related to hypertension, and persons with lower education are more likely to be diagnosed with MCI, then it is possible that the relation between hypertension and all-cause or non-amnestic MCI could be due to confounding by socioeconomic factors. We adjusted for years of education and ethnicity as markers of socioeconomic status to account for this possibility. However, it is possible that hypertension is related to other behaviors related to poor health, that in turn may increase the risk of cognitive decline that we could not adjust for, and we cannot eliminate the possibility of lack of control for unknown confounders as a potential explanation for our findings.

The main limitation of our study is the lack of subclinical markers of hypertension, such as left ventricular hypertrophy by EKG or echocardiogram, and the use of self reported history as our main measurement of hypertension. As shown in our sample, most elderly people will develop hypertension in their lifetime.[44] Therefore, elderly cohorts may be too homogeneous to show differences in outcomes related to a history of hypertension. Our measurement of hypertension did not take into account severity or duration. Thus, it is possible that our results tend to underestimate the association between hypertension and MCI, and could bias our results to the finding of no association with amnestic MCI. It is possible that studies in younger age groups with measures of hypertension burden in mid-life could find stronger associations with risk of MCI than we report, including an association with amnestic MCI. Also, it is important to point out that this study was conducted in an elderly multiethnic community in an urban setting with a high prevalence of risk factors for morbidity and mortality, such as

diabetes and hypertension. Persons who dropped out of the study during follow-up were at baseline older, less educated and had a higher prevalence of vascular risk factors than those who remained in the study. Also, hypertension is related to higher cardiovascular mortality, and it is possible that some hypertensive persons would have demonstrated cognitive decline had they not died prior to inclusion in this cohort. Thus, there are important biases related to the sample of this study that should be taken into account in the interpretation and generalization of these findings. We did not have information on brain magnetic resonance imaging and measures of cerebrovascular disease. Thus, our stroke variable is likely an underestimation of the prevalence of cerebrovascular disease. We expected that the other vascular risk factor variables would be surrogate markers of cerebrovascular disease risk. Our ascertainment of MCI subtypes was based on neuropsychological criteria and would not have been affected by the availability of imaging data.

The main strength of our study is that it is a prospective cohort study designed for the diagnosis of cognitive impairment and dementia with standard criteria, and with complete clinical and neuropsychological evaluation at each interval that permitted the ascertainment of different types of incident MCI.

Our findings suggest that hypertension increases the risk of incident MCI, especially non-amnestic MCI. The importance of non-amnestic forms of cognitive impairment are increasingly recognized,[40,45] and preventing and treating hypertension is likely to have an important impact in lowering the risk of cognitive impairment.

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3

Genetic polymorphisms and risk of dementia and cerebral small- vessel disease

3.1

CRP-gene haplotypes, serum CRP, and cerebral small-vessel disease

ABSTRACT

Objective. C-reactive protein (CRP), an acute phase protein reflecting an inflammatory condition, has been repeatedly associated with cardiovascular and cerebrovascular disease, including subortical and periventricular white matter lesions (WML). However, whether CRP is a direct causal factor in the pathogenesis of atherosclerosis, or merely a serum marker for atherothrombotic disease, remains unclear. In this study we sought to explore this question by studying the association between known variations in the CRP gene, which have been consistently associated with serum CRP-levels, and cerebral small-vessel disease (SVD), in two independent cohort studies, the Rotterdam Scan Study (n=1035) and the 'Memory and Morbidity in Augsburg Elderly' (MEMO) Study (n=268). **Methods.** Common haplotypes within the CRP gene were determined by genotyping tagging SNPs. Then their relation with periventricular and subcortical WML and prevalence of lacunar brain infarcts was explored using ANOVA and linear and logistic regression analyses. Also examined were the interactions between serum CRP, CRP gene polymorphisms and WML and brain infarcts, respectively. **Results.** We found the expected associations between CRP haplotypes and serum CRP levels, which were consistent in both studies. However, there was no relation between haplotypes and measures of cerebral SVD in either study. There was also no effect modification of the association between serum CRP levels and measures of SVD by CRP haplotypes. **Conclusion.** Our observations in two separate cohort studies do not suggest that CRP is causally involved in the pathogenesis of cerebrovascular small vessel disease. More insight into the exact mechanisms underlying the association between CRP and vascular disease is necessary.

INTRODUCTION

Inflammatory processes are implicated in the pathogenesis of atherosclerosis. Inflammation promotes atherosclerosis and thrombosis by elevating serum levels of fibrinogen,[1] leukocytes,[2] clotting factors,[3] and cytokines.[4] On a cellular level inflammatory processes promote atherosclerosis by altering the metabolism and functions of endothelial cells and monocyte macrophages.[5]

C-reactive protein (CRP), an acute-phase reactant, is an indicator of an inflammatory condition of blood vessels and a serum marker for atherothrombotic disease.[5] A strong and consistent association between clinical manifestations of atherothrombotic disease and baseline CRP levels has been described in patients with angina pectoris,[6] myocardial infarction,[7,8] stroke[7-12] and peripheral arterial disease.[7,13]

However, whether CRP is only a marker of severity of vascular disease or actually plays a pathogenic role in vascular disease development, remains unclear. The previously reported associations between CRP levels and vascular disease may have been caused by residual confounding and thus might not be causal.[14] Study of the association between genetic variation in the CRP gene and vascular disease may help to further elucidate the role of CRP in vascular disease taking residual confounding into account. Alleles of the CRP gene influencing CRP levels are transmitted from parent to offspring at random, and thus factors that may confound associations between CRP levels and vascular disease are likely to be evenly distributed among those with and without alleles causing high CRP levels.[14] If CRP is a causal factor in the development of atherosclerosis, vascular disease must not only be associated with CRP serum levels but also with genetic variation in the CRP gene.

Seattle SNPs (National Heart Lung and Blood Institute's Programs for Genomic Applications) has identified four CRP gene haplotypes in populations of European descent, which represent all common variation across the CRP gene in these populations. To further clarify the role of CRP in vascular disease, we sought to examine in the present study whether these four CRP haplotypes are associated with the risk of cerebral small-vessel disease (SVD), which has been associated with increased serum CRP levels in the Rotterdam Scan Study in previous analyses.[15]

We did this in the two independent population-based cohorts of the Rotterdam Scan Study and the 'Memory and Morbidity in Augsburg Elderly' (MEMO) Study.

METHODS

Participants and Setting. The *Rotterdam Scan Study* is a prospective, population-based cohort study to which 1,077 participants, aged 60 to 90 years, were randomly selected from 2 large ongoing population-based studies.[16] The baseline examination in 1995 to 1996 comprised a structured interview, neuropsychological tests, physical examination, blood sampling, and an MRI scan of the brain. In 1999 to 2000, 668 (70%) of the 951 participants who were alive and eligible underwent a second MRI. In the *MEMO Study* enrolled were 385 participants, 65 years or older, and living in the city of Augsburg, Germany. [17] Each participant underwent an in-person interview of medical history and current medication, a standardized neurological examination, neuropsychological tests, blood sampling and an MRI of the brain. Both studies had been approved by the local medical ethics committees.

From the 1,077 participants in the *Rotterdam Scan Study* who underwent baseline examination, genotyping was unavailable in 39 (3.6%) subjects, and 3 persons (0.3%) were excluded due to genotyping errors. In the *MEMO Study*, 118 participants (30.6%) could not undergo the MRI assessment due to contraindications. Thus, the final analytic samples in the Rotterdam Scan Study comprised 1,035 participants, of which 636 underwent a second MRI at follow-up, and in the MEMO Study 268 participants.

Magnetic Resonance Imaging (MRI). In both studies, MRI scans were performed using 1.5 tesla machines (MR VISION, Siemens; MR Gyroscan, Philips). The standardized MRI protocol included proton density (PD), T1 and T2 weighted images acquired with spin echo sequences with 20 axial slices, 5 mm thick, with an interslice gap of 1 mm. For both studies, two raters, blinded for all clinical information, independently analyzed the images for periventricular and subcortical WML and cerebral infarcts using an established rating scale.[18]

White matter lesions (WML). WML were considered to be in the periventricular region if they were directly abutting the lateral ventricle; otherwise they were considered subcortical. Periventricular WML were graded semiquantitatively on a severity scale (0–3) at the frontal and occipital horns and the body of the lateral ventricle, with the total periventricular WML score being the sum of these three scores. For subcortical WML the total volume was approximated based on number and size of lesions (range 0 to 30.0 mL).

Two raters independently assessed progression of white matter lesion severity on digital T2-weighted and PD-weighted images by direct scan comparison.[19] Raters were blinded to all clinical information. We scored differences in white matter lesion severity in the 3 periventricular regions of both hemispheres (periventricular score range –6 to 6) and in the subcortical white matter of the 4 lobes of both hemispheres (subcortical score range –8 to 8).[19] The change rating showed good interobserver agreement (intraclass correlation coefficient 0.75

to 0.79) and good to very good intraobserver agreement (intraclass correlation coefficient 0.70 to 0.93). If raters disagreed by 1 point or less on the scale, we used the mean of the ratings; otherwise, we held a consensus meeting. Adjudication by consensus meeting was required in 9% of the periventricular and 11% of the subcortical white matter lesion ratings. Progression was defined as an increase of 1 point or more between baseline and follow-up. We categorized progression into categories of no progression (score <1), minor progression (score 1 to 2.5), and marked progression (score ≥3). Hyperintensities on PD- and T2-weighted images around an incident infarct were not considered as progression of white matter lesions.

Cerebral infarcts. Infarcts were defined as areas of focal hyperintensity on T2-weighted images with a diameter ≥ 3 mm. Lacunar infarcts were defined as infarcts sized 3-15 mm and located in the subcortical white matter or basal ganglia.

Serum CRP levels and Cardiovascular Risk Factors. In both studies serum levels of CRP were determined by high sensitivity rate near infrared particle immunoassay method (Immage high-sensitivity CRP, Beckman Coulter). Hypertension was defined according to World Health Organization-International Society of Hypertension guidelines at time of blood pressure measurement as systolic blood pressure ≥ 160 mm Hg, diastolic blood pressure ≥ 100 mm Hg, or the use of blood pressure–lowering medication. Smoking habits were classified as never, former, or current cigarette smoking. Diabetes mellitus was considered to be present if the random glucose level was ≥11.1 mmol/L or if a person had a history of diabetes or was taking oral antidiabetic medications. Body mass index was calculated as weight divided by height squared.

Genotyping. The Seattle SNPs Program for Genomic Applications has, based on 23 unrelated individuals of European descent from the CEPH pedigrees, identified 31 SNPs in the CRP gene. Based on all SNPs with overall frequencies above 5%, it subsequently identified four common CRP gene haplotypes to be present in populations of European descent. We genotyped three haplotype tagging SNPs to infer these four haplotypes.[20]

In the *Rotterdam Scan Study*, DNA was genotyped for 1184C>T, 2042C>T, and 4741C>G polymorphisms (Seattle SNPs, http://pga.gs.washington.edu). In the *MEMO Study* genotyping was performed for 1184C>T, 2042C>T, and 4363C>A polymorphisms, also tagging the same four haplotypes. The 4363C>A tagging SNP is in perfect linkage disequilibrium with the 4741C>G polymorphism assessed in the Rotterdam Scan Study. The polymorphisms are described in relation to the start of the coding sequence of exon 1 using the Human May 2004 (hg 17) assembly (http://genome.ucsc.edu). These polymorphisms have also been described at http://www.ncbi.nlm.nih.gov/SNP under identification numbers rs1130864 (1184C>T), rs1205 (2042C>T),

rs3093068 (4741C>G), and rs3093075 (4363C>A). Using the HapMap website (http://www.hapmap.org), they were found to lie in one linkage disequilibrium block.

In both studies, DNA was isolated using standard procedures. Genotypes were determined in 2-ng genomic DNA with Taqman allelic discrimination assays (Applied Biosystems). Reactions were performed with the Taqman Prism 7900HT 384-wells format in 5 µL of reaction volume. Haplotype alleles present in the population were inferred by means of the haplo.em function of the program Haplo Stats (http://cran.r-project.org/src/contrib/ Descriptions/ haplo.stats.html), which computes maximum likelihood estimates of haplotype probabilities.[21,22] In both studies haplotype reconstruction resulted in six haplotypes, but the fifth and sixth haplotypes were present in <0.001% of the alleles and were therefore not used in the analyses. Haplotype alleles were coded as haplotype numbers 1 through 4 in order of decreasing frequency in the population: coding from 1184C>T, 2042C>T and 4741 C>G (4363C>A), haplotype 1= C-A-C, 2= C-G-C, and 3= T-G-C. Haplotype 4 was in the Rotterdam Scan Study coded C-G-G and in the Memo Study C-G-A.

Statistical Methods. First, in both study populations Hardy-Weinberg equilibrium of the CRP polymorphisms was assured using a χ^2 test. Then the genotypic distributions and baseline and clinical characteristics in both studies were evaluated. We then compared the mean serum CRP levels among the genotypes of each polymorphism using analysis of variance (ANOVA). Since the distribution of CRP levels was skewed, logarithmic transformation of this variable was carried out before analyses were performed.

To assess the association between the individual polymorphisms and measures of cerebral SVD, we compared the mean grades of periventricular and subcortical WML severity among the genotypes of each individual polymorphism using ANOVA, and estimated the association of each individual polymorphism with the prevalence of lacunar brain infarcts (no/any) using multivariate logistic regression analyses, adjusting all models for age and sex. To explore the effect modification of the association between serum CRP levels and cerebral SVD by CRP gene polymorphisms, we subsequently repeated all analyses adding an interaction term to the models that contained variables for the genotypes of the individual polymorphisms (carriers of the mutant allele vs. non-carriers) and serum CRP levels. In the Rotterdam Scan Study, we additionally assessed the interaction between serum CRP levels, CRP gene polymorphisms and progression of WML with binomial logistic regression analyses (none/any progression) and with multinomial logistic regression analyses (any/minor/marked progression). We also performed binomial logistic regression analyses to explore the interaction between CRP gene polymorphisms, CRP levels and incident lacunar infarcts. We performed all analyses with CRP levels categorized in quartiles of the baseline distribution, and with CRP levels as a logarithmic transformed continuous variable. After adjusting for age and sex we additionally adjusted for diabetes, smoking, body mass index, and hypertension in subsequent models.

To test the associations of CRP gene haplotypes with measures of cerebral SVD, we used the program Haplo.Stats, which is implemented in the R software (http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html).[21-23] The probability for each haplotype pair in each individual was assigned and then an individual's phenotype was directly modeled as a function of each inferred haplotype pair, weighed by their estimated probability, to account for haplotype ambiguity. The haplo.score function of Haplo.Stats was used to test the associations.[23] We adjusted for age and sex and we computed global simulation P-values and simulation P-values for each haplotype. The number of simulations was set as 1000.

The association between CRP gene haplotypes and CRP serum level, and measures of SVD was investigated by using the haplo.glm function of Haplo.Stats, adjusting all models for age and sex.[22] This approach is based on a generalized linear model, and computes the regression of a trait on haplotypes and other covariates. For the analysis regarding the disease outcomes, the haplotype that was associated with the lowest serum CRP levels served as the reference category.

RESULTS

The genotypic distribution and demographic and clinical characteristics of the participants in the Rotterdam Scan Study and MEMO Study are shown in table 1. Genotype distributions for all tagging SNPs were in Hardy-Weinberg equilibrium.

In the Rotterdam Scan Study persons who were hetero- or homozygeous for the 1184T or 4741G minor allele had significantly higher serum CRP levels than persons of similar age and gender who were homozygeous for the common allele (adjusted means for serum CRP levels: 3.9 vs. 3.0 mg/l, p= 0.02, and 4.7 vs. 3.4 mg/l, p=0.04, respectively). Carriers of at least one 2042T minor allele had significantly lower serum CRP levels than non-carriers (adjusted mean 0.4 vs. 3.1 mg/l, p=0.02). Similarly, in the MEMO Study carriers of the minor 2042T allele had significantly lower CRP serum levels than non-carriers (adjusted mean 3.0 vs. 3.9 mg/l, p=0.05), and there was an association between the 4363A allele and higher CRP levels that was close to statistical significance (adjusted mean for CRP levels: 3.8 vs. 3.1, p= 0.08).

Relating the genotypes of the individual polymorphisms with the grades of periventricular and subcortical WML, there were no differences in mean severity of WML among the genotypes of any polymorphism (table 2). There was also no association between carrier status of any polymorphism and the presence of lacunar infarcts (table 3), or between any polymorphism and grade of progression of SVD.

To assess the effect modification of the association between serum CRP levels and cerebral SVD by the individual CRP-gene polymorphisms, we then repeated all analyses adding the interaction term to the models that contained variables for the genotypes of the individual

Table 1. Genotype distributions and demographic and clinical characteristics in the Rotterdam Scan Study and the MEMO Study

	Rotterdam Scan Study (n=1035)	MEMO Study (n=268)
Women, n (%)	549 (51.9)	124 (47.1)
Mean age (years)	72.3 (7.4)	72.2 (4.4)
APOEε4 -/4 or 4/4 genotype, n (%)	260 (24.6)	74 (28.1)
Body mass index, mean (SD)	26.7 (3.6)	27.8 (3.6)
Diabetes mellitus, n (%)	75 (7.1)	23 (8.6)
Hypertension, n (%)	551 (52.1)	127 (47.4)
Current smoking, n (%)	182 (17.3)	26 (9.7)
CRP serum level (mg/l), mean (SD)	3.5 (6.1)	3.5 (5.7)
Grade of periventricular white matter lesions, mean (SD) *	2.4 (2.2)	1.8 (1.9)
Volume of subcortical white matter lesions (ml), mean (SD) †	1.4 (2.9)	1.5 (2.4)
Lacunar infarcts, n (%)	212 (20.1)	40 (15.2)
1184C>T polymorphism genotype, n (%)		
CC	498 (47.1)	127 (52.5)
CT	443 (41.9)	96 (39.7)
TT	90 (8.5)	19 (7.8)
2042C>T polymorphism genotype, n (%)		
GG	439 (41.5)	103 (47.5)
GA	489 (46.3)	93 (42.9)
AA	107 (10.1)	21 (9.7)
4741C>G (4363C>A) polymorphism genotype, n (%) ‡		
CC (CC)	926 (87.6)	231 (91.3)
CG (CA)	96 (9.1)	22 (8.7)
GG (AA)	9 (0.9)	-/-

* periventricular WML were graded semiquantitatively on a severity scale (0–3) at the frontal and occipital horns and the body of the lateral ventricle. The total periventricular WML score is the sum of these three scores. † the total volume of subcortical WML was approximated based on number and size of lesions (range 0 to 30.0 mL). ‡4741C>G and 4363C>A polymorphisms are in perfect linkage disequilibrium. 4741C>G polymorphism was genotyped in the Rotterdam Scan Study, 4363C>A polymorphism was genotyped in the MEMO Study

polymorphisms and serum CRP levels. In these analyses, there was no effect modification of the association between serum CRP levels and measures of SVD by any polymorphism in either study.

Haplotypes were in the Rotterdam Study present in the following frequencies: haplotype 1 (C-A-C): 33.9%; haplotype 2 (2= C-G-C): 30.4%, haplotype 3 (T-G-C): 30.1%, and haplotype 4 (C-G-G): 5.4%. In the MEMO Study the frequencies were as follows: haplotype 1 (C-A-C): 33.9%; haplotype 2 (C-G-C): 31.6 %, haplotype 3 (T-G-C): 30.0%, haplotype 4 (C-G-A): 4.1%. In both studies, haplotypes 2, 3, and 4 were associated with higher CRP serum levels than haplotype 1, therefore the latter served as reference category in further analyses.

Table 2. Comparison of periventricular and subcortical WML severity* across genotypes of CRP gene polymorphisms

Study	1184C>T		2042C>T		4741C>G (4363C>A) †	
	CC	CT or TT	GG	GA or AA	CC (CC)	CG or GG (CA or AA)
Rotterdam Scan Study						
Periventricular WML	2.38 (0.09)	2.36 (0.09)	2.31 (0.09)	2.41 (0.08)	2.40 (0.06)	2.12 (0.19)
Subcortical WML	1.32 (0.12)	1.42 (0.12)	1.41 (0.13)	1.34 (0.11)	1.37 (0.09)	1.38 (0.27)
MEMO Study						
Periventricular WML	1.61 (0.02)	1.91 (0.18)	1.96 (0.19)	1.55 (0.18)	1.73 (0.12)	2.35 (0.40)
Subcortical WML	1.50 (0.21)	1.41 (0.22)	1.25 (0.22)	1.57 (0.21)	1.51 (0.16)	1.39 (0.51)

*Adjusted means (SE) of periventricular and subcortical WML severity, derived from analysis of variance (ANOVA) adjusted for age and gender
† 4741C>G and 4363C>A polymorphisms are in perfect linkage disequilibrium. 4741C>G polymorphism was genotyped in the Rotterdam Scan Study, 4363C>A polymorphism was genotyped in the MEMO Study

Table 3. Odds ratios and 95% confidence intervals, relating carrier status of CRP polymorphisms (carriers of minor allele vs. non-carriers) and the risk of lacunar brain infarcts (any vs. no infarct)

	Rotterdam Scan Study OR (95% CI)	MEMO Study OR (95% CI)
1184C>T		
CC	1.0	1.0
CT or TT	1.1 (0.81-1.53)	1.7 (0.85-3.71)
2042C>T		
AA	1.0	1.0
GA or GG	0.9 (0.65-1.23)	0.8 (0.37-1.69)
4741C>G (4363C>A) *		
CC (CC)	1.0	1.0
CG or GG (CA or AA)	0.9 (0.58-1.71)	1.6 (0.59-5.11)

Logistic regression. OR= odds ratio; 95% CI= 95% confidence interval. All models are adjusted for age and gender. * 4741C>G and 4363C>A polymorphisms are in perfect linkage disequilibrium. 4741C>G polymorphism was genotyped in the Rotterdam Scan Study, 4363C>A polymorphism was genotyped in the MEMO Study.

In analyses relating haplotypes with measures of SVD, haplotype 3 was in the MEMO Study associated with a higher severity grade of subcortical WML than haplotype 1 after adjusting for age and gender (β 0.57, SE 0.26, p=0.04, table 4). However, taking adjustment for multiple comparisons into account this is a non-significant finding. There was no association between any other haplotype and subcortical WML, and there was no association between haplotypes and periventricular WML or lacunar brain infarcts in either study (tables 4 and 5). There was also no effect modification of the association between serum CRP levels and measures of SVD by CRP haplotypes in either study.

Table 4. Age- and sex-adjusted ß-coefficients (SE) relating CRP haplotypes with periventricular and subcortical WML

	Rotterdam Scan Study		MEMO Study	
	Periventricular WML ß (SE)	Subcortical WML ß (SE)	Periventricular WML ß (SE)	Subcortical WML ß (SE)
Haplotype 1 (C-A-C)	reference	reference	reference	reference
Haplotype 2 (C-G-C)	-0.09 (0.11)	0.11 (0.16)	-0.19 (0.22)	0.11 (0.26)
Haplotype 3 (T-G-C)	-0.11 (0.11)	0.09 (0.16)	1.17 (0.21)	0.57 (0.26)*
Haplotype 4 (C-G-G / C-G-A) †	-0.27 (0.19)	0.08 (0.28)	0.01 (0.43)	0.05 (0.54)

* significant at a 0.05 level. † Haplotype 4 was coded C-G-G in the Rotterdam Scan Study and C-G-A in the Memo Study.

Table 5. Age- and sex-adjusted Odds Ratios (95% CI) relating CRP haplotypes with lacunar brain infarcts

	Rotterdam Scan Study OR (95% CI)	MEMO Study OR (95% CI)
Haplotype 1 (C-A-C)	reference	reference
Haplotype 2 (C-G-C)	1.2 (0.87-1.55)	1.0 (0.53-1.78)
Haplotype 3 (T-G-C)	1.1 (0.83-1.48)	1.0 (0.53-1.83)
Haplotype 4 (C-G-G / C-G-A) *	1.0 (0.57-1.66)	1.2 (0.38-3.86)

* Haplotype 4 was coded C-G-G in the Rotterdam Scan Study and C-G-A in the Memo Study.

DISCUSSION

We found an association between the 1184T or 4741G minor allele and higher serum CRP levels in the Rotterdam Scan Study, and an association of the 2042T minor allele with lower CRP levels in both the Rotterdam Scan Study and the MEMO Study. We also found associations between CRP haplotypes and CRP serum levels, which were consistent in both studies. There was no relation between any individual polymorphism and WML or lacunar infarction in either study, and there was no effect modification of the association between serum CRP levels and measures of cerebral SVD by any polymorphism. There was no association between CRP haplotypes and SVD in either study.

Our study has important strengths. It was based on two independent population based studies, and diagnoses of cerebral lesions were made using the same standardized MRI reading protocol in both studies.

Limitations of the study include that non-participation was in both the Rotterdam Scan Study and the MEMO Study associated with older age and a higher prevalence of vascular risk factors. There is a possibility that such selective attrition leads to an overestimation of the associations between polymorphisms and SVD since genetic contribution to disease is less in older age.

However, since we did not observe an association, a selection effect seems unlikely. Second, although two raters independently assessed all MRI images with good interrater agreement, there remains a possibility of misclassification of brain lesions. Also, because genotypes were assessed only once, there is room for genotyping errors. Assuming non-differential misclassification, such errors would have led to an underestimation of the true association between CRP polymorphisms and SVD.

To account for the complete common genetic variation of the CRP gene in our analyses, we did not only assess the association of individual polymorphisms with SVD, but used also haplotypes describing the total CRP gene variation. Only few other studies have used this approach before. Carlson et al.,[24] who defined the common genetic variation across the CRP gene by resequencing the region in 24 African Americans and 23 European Americans, found an association between their haplotypes 5 and 7 with higher CRP levels, and haplotypes 1 and 2 with lowest CRP levels. These results are in agreement with the findings in our corresponding haplotypes. Miller et al.[25] ascertained a comprehensive set of common variants in the CRP gene by resequencing 192 individuals of the Physicians health Study (PHS). They then studied and replicated the association of these variants with baseline CRP levels in apparently healthy subjects in the Women's Health Study (WHL), Pravastatin Inflammation/CRP Evaluation trial (PRINCE) and PHS, and also assessed their association with myocardial infarction and stroke in a nested case-control study within the PHS. They found a haplotype pattern consistent with the haplotype pattern of Seattle SNPs, and their results were again in agreement with ours.

We did not find a direct association between CRP polymorphisms or CRP haplotypes and measures of cerebral SVD, although we observed an association of individual polymorphisms and CRP haplotypes with plasma CRP levels, which in turn have been consistently associated with vascular disease in observational studies,[6,26-29] and were also associated with cerebral SVD in the same sample of the Rotterdam Scan Study in previous analyses.[15] Because CRP is associated with several other risk factors, relations between CRP and vascular disease observed in previous studies might not be causal but caused by residual confounding.[14] The approach used in the present study, which has also been called "Mendelian randomization", overcomes this problem, since alleles of the CRP gene influencing CRP levels are transmitted from parent to offspring at random, and thus factors that may confound associations between CRP levels and cerebral SVD are likely to be evenly distributed among those with and without alleles causing high CRP levels.[14]As a consequence, our observations of no association between genetic variation in the CRP gene and measures of cerebral SVD, which take possible residual confounding into account, do not suggest a causal role of CRP in the pathogenesis of vascular disease.

One might consider alternative explanations of our findings. First, it is possible that our study lacked statistical power to detect a small effect size. Power calculation for the Rotterdam Scan Study shows that, with a power of 80% and an alpha of 0.05, in reference to haplotype 1, (the most common haplotype, frequency 33.9%), we were able to demonstrate relative risks for lacunar brain infarcts of at least 1.45 (for haplotype 2, frequency 30.4%). If there indeed is an association between CRP gene haplotypes and cerebral SVD, it must be of relatively small magnitude. Second, inflammation might be a response rather than cause of ischemic tissue damage.[30] However, the approach chosen in the present study also overcomes reverse causation since genotypes are determined before onset of disease and do not change during life.[14]

In summary, this study of two separate cohorts does not suggest that CRP plays a causal role in the pathogenesis of cerebrovascular disease. More insight into the exact mechanisms underlying the association between serum CRP levels and vascular disease is needed.

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3.2

Matrix Metalloproteinase 3 Haplotypes, Dementia and Hippocampus Volume

ABSTRACT

Evidence by post-mortem and animal studies suggests that matrix metalloproteinases (MMPs) may play an important role in the pathophysiology of Alzheimer's disease (AD) through degradation of amyloid beta. We investigated in 5,999 elderly whether MMP3- haplotypes are associated with dementia and AD. We also explored the association of MMP-3 haplotypes with changes in hippocampus volume and severity of periventricular and subcortical white matter lesions (WML). There was no association between any individual polymorphism or MMP-3 haplotypes and dementia or AD. In analyses relating the genotypes of the individual polymorphisms with hippocampus volume, carriers of the 5A allele of the 5A6A promotor polymorphism had a smaller hippocampus volume compared with persons who were homozygeous for the 6A allele. There was no association between MMP-3 haplotypes and hippocampus volume, and there was no relation between MMP3-genotypes or -haplotypes with severity of periventricular or subcortical WML. These associations did not differ between strata of APOEε4 genotype. Our observations do not suggest that variation in the MMP3 gene is causally involved in dementia or AD.

INTRODUCTION

Alzheimer's disease is the most common form of dementia in western societies. Its main pathological hallmark is the presence of senile plaques with aggregation of Amyloid ß (Aß). Although the mechanism of Aß generation is well understood, the exact pathways of its degradation remain unclear.

Findings of recent animal and post mortem studies suggest that matrix metalloproteinases (MMPs), a family of zinc- and calcium-dependent endopeptidases that are involved in the degradation of connective tissue and extracellular matrix (ECM), are implicated in the pathogenesis of AD. MMP-3 (Stromelysin-1) might in particular play a central role because it activates several latent-type MMPs such as MMPs -1, -8, -9 and -13, which in turn are involved in Aß degradation.[1,2] MMP-3 is also directly involved in Aß degradation,[1,2] and there is evidence that it has a reduced expression in AD hippocampi,[3] suggesting that it plays a role in selective neurodegeneration. MMP-3 is also involved in the pathogenesis of atherosclerosis; common polymorphisms in the gene encoding MMP-3, in particular the 5A6A promotor polymorphism, have been repeatedly associated with the risk of atherosclerosis and cardiovascular disease,[4-8] which in turn have been related with the risk of AD in several studies.[9-11]

Three studies explored the association between variation in genes encoding MMPs and dementia in a population-based observational setting.[12-14] All assessed, in a cross-sectional design, the impact of individual single nucleotid polymorphisms (SNPs) on the frequency of dementia. None of the studies took the complete common genetic variation into account, and none of the studies assessed the association of genetic variation in MMP genes with risk of dementia or AD in a longitudinal manner.

The Seattle SNPs Program for Genomic Applications (http://pga.gs.washington.edu) has, based on 23 unrelated individuals of European descent from the CEPH pedigrees, identified 41 SNPs in the gene encoding MMP-3. By genotyping three tagging SNPs with overall frequencies above 4%, we inferred four haplotypes representing the complete genetic variation in the MMP-3 gene in populations of European descent.

The objective of the present study was to assess the association of these common haplotypes with the risk of dementia and AD in the large population-based sample of the Rotterdam Study. We also sought to assess the association of variation in the MMP-3 gene with differences in hippocampus volume and severity of periventricular and subcortical white matter lesions (WML) in the Rotterdam Scan Study. Decreased hippocampus volume and a higher burden of WML are neuropathological changes potentially underlying dementia.

METHODS

Participants and Setting. The Rotterdam Study is a population-based prospective cohort study that was designed to investigate the incidence and causes of cardiovascular, neurodegenerative, locomotor, and ophthalmologic diseases in the elderly.[15] From 1990 to 1993, all 10,275 residents aged ≥ 55 years of Ommoord, a district of the city of Rotterdam, were invited to participate, and 7,983 (78%) men and women agreed. The Medical Ethics Committee of the Erasmus Medical Center approved the study, and written informed consent was obtained from all participants. During the baseline examination (1990-1993), a research assistant interviewed participants in their homes and obtained information on current and past health, medication, lifestyle, and risk factors for chronic diseases. In addition, participants visited the research center twice for baseline clinical examinations. Follow-up examinations took place in 1993-1994, 1997-1999 and 2002-2004. Through linkage with records of general practitioners, the entire cohort was continuously monitored for morbidity and mortality.

From the 7,983 participants who underwent baseline examination, 7,528 were screened for dementia (94.3%). From these, 482 persons (6.4%) were diagnosed with prevalent dementia, and 1,074 (14.3%) persons missed information on MMP-3 genotyping. The final analytic sample included in this study comprised 5,999 persons without dementia at baseline and with complete information on MMP-3 genotypes.

Diagnosis of Dementia and Alzheimer Disease. Diagnostic procedures for dementia and Alzheimer disease have been described in detail.[16] At baseline and both follow-up examinations, a three-stage protocol was used to screen all participants cognitively with the Mini-Mental State Examination (MMSE)[17] and the Geriatric Mental State schedule (GMS) organic level.[18] If subjects scored lower than 26 on the MMSE or higher than 0 on the GMS organic level, the Cambridge Examination of Mental Disorders in the Elderly (CAMDEX)[19] was administered. The CAMDEX also included an informant interview. Finally, participants in whom dementia was suspected were examined by a neurologist and neuropsychologist and, if possible, underwent magnetic resonance imaging of the brain. In addition, the total cohort was continuously monitored for incident dementia cases through computerized linkage between the study database and computerized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.[16] The diagnoses of dementia and Alzheimer disease were based on *Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition* (*DSM-III-R*) criteria[20] and the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria,[21] respectively, and were made by a panel of a neurologist, neuropsychologist, and research physicians who reviewed all existing information.[16] Follow-up with respect to dementia was nearly complete (99.9%).

Hippocampus volume. From 1995 to 1996, 965 living members (aged 60-90 years) of the Rotterdam Study were randomly selected in strata of sex and age (5 years) for participation in the Rotterdam Scan Study, a study on age-related brain changes on MRI.[22] As part of the eligibility criteria, we excluded individuals who had dementia, or had MRI contraindications.[22] This left 832 persons eligible for participation. Among these, 563 (68%) persons gave their written informed consent to participate in the present study. Complete MRI data, including a 3-dimensional MRI sequence, were obtained in 511 persons.[23] The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, The Netherlands.

At baseline examinations from 1995 to 1996, a 3-dimensional MRI sequence covering the whole brain was made using a 1.5-T MRI unit.[23] We reformatted coronal slices (1.5-mm contiguous slices) from this 3-dimensional MRI sequence in such a way that they were perpendicular to the long axis of the hippocampus. The left and right hippocampus and amygdala were manually outlined on each slice with a mouse-driven cursor. Absolute volumes were calculated by multiplying the areas on each slice by the slice thickness. We summed the left and right sides to yield total volumes because the analyses did not suggest laterality of effects. As a proxy for head size, we measured the intracranial cross-sectional area on a reformatted middle sagittal MRI slice. Two readers who were blinded to clinical information measured the 511 images. Intrarater and interrater correlation coefficients have been reported and showed good reproducibility.[23] We corrected for head size differences across individuals by dividing the raw volumes by the subject's calculated head size and subsequently multiplying this ratio by the average head size area, separately for men and women.[24]

White matter lesions (WML). WML were considered to be in the periventricular region if they were directly abutting the lateral ventricle; otherwise they were considered subcortical. Periventricular WML were graded semiquantitatively on a severity scale (0–3) at the frontal and occipital horns and the body of the lateral ventricle, with the total periventricular WML score being the sum of these three scores. For subcortical WML the total volume was approximated based on number and size of lesions (range 0 to 30.0 mL).

Assessment of covariates. At baseline, trained investigators interviewed all participants at home, collecting information on socioeconomic status, current health status and medical history. In addition, clinical measures were obtained at the research center. Level of education was categorized into 3 groups: low (primary education only); intermediate (lower vocational or general education); and high (intermediate or higher vocational or general education, college, or university). Smoking habits were categorized as ever smoking and non-smoking. Body mass index was calculated using the formula [weight (kg)/length (m^2)]. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. Diabetes mellitus was defined as a random or postload glucose

level ≥ 11.1 mmol/L or a history of diabetes or the use of blood glucose-lowering medication.

Nonfasting blood samples were drawn and immediately frozen. Total cholesterol, high-density lipoprotein cholesterol, and glucose were measured within 2 weeks, as described previously.[25] Levels of serum C-reactive protein (CRP) were determined by the rate near infrared particle immunoassay method (Immage high-sensitivity CRP, Beckman Coulter).

Furthermore, ultrasonography of both carotid arteries was performed. As an indicator of atherosclerosis of the carotid arteries, we used intima media thickness (IMT). Common carotid IMT was determined as the average of the maximum IMT of near- and far-wall measurements, and the average of left and right common carotid IMT was computed.[26] Apolipoprotein E (APOE) genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis.[27] After excluding persons with the APOEε2/ε4 genotype, we dichotomized APOE genotype into presence or absence of the apolipoproteinEε4 (APOEε4) allele.

Genotyping. The Seattle SNPs Program for Genomic Applications has, based on 23 unrelated individuals of European descent from the CEPH pedigrees, identified 41 SNPs in the gene encoding MMP-3. By genotyping three tagging SNPs with overall frequencies above 4%, we were able to infer four haplotypes representing the complete genetic variation in the MMP-3 gene in populations of European descent.

DNA was genotyped for 1187 (5A6A), 2092A>G, and 9775T>A polymorphisms (Seattle SNPs, http://pga.gs.washington.edu). These polymorphisms have also been described at http://www.ncbi.nlm.nih.gov/SNP under identification numbers rs3025058 (1187 (5A6A)), rs522616 (2092A>G), and rs563096 (9775T>A).

DNA was isolated using standard procedures. Genotypes were determined in 2-ng genomic DNA with Taqman allelic discrimination assays (Applied Biosystems). Reactions were performed with the Taqman Prism 7900HT 384-wells format in 5 µL of reaction volume. Haplotype alleles present in the population were inferred by means of the haplo.em function of the program Haplo Stats (http://cran.r-project.org/src/contrib/ Descriptions/ haplo.stats.html), which computes maximum likelihood estimates of haplotype probabilities.[28-30] Haplotype reconstruction resulted in four haplotypes with a frequency of > 0.001% of the alleles. Haplotype alleles were coded as haplotype numbers 1 through 4 in order of decreasing frequency in the population: coding from 1187 (5A6A), 2092A>G and 9775T>A, haplotype 1= 5A-A-T, 2= 6A-G-T, 3= 6A-A-T, and 4=6A-A-A.

Statistical Methods. First, Hardy-Weinberg equilibrium of the MMP-3 polymorphisms was tested using a χ^2 test. Then the genotypic distributions and baseline and clinical characteristics were evaluated.

Cox proportional hazards models were used to assess the association between the individual polymorphisms and risk of incident dementia and AD in persons free of dementia at baseline, adjusting all models for age and sex. The time-to-event variable in these analyses was age at onset of dementia and AD, death or end of follow-up, respectively. Persons who did not develop dementia, who died, or who were lost to follow-up owing to relocation before development of dementia were censored at the time of their last evaluation. Follow-up with respect to dementia was nearly complete (99.9%). Multivariate linear regression analyses were used to estimate the association of each individual polymorphism with hippocampus volume and severity of periventricular and subcortical WML, respectively. Since the MMP-3 gene is located on chromosome 11q22.3, a region previously linked with AD particularly in APOEε4 non-carriers,[31] we finally repeated all analyses stratifying by APOEε4 genotype. As described above, carriers of the APOEε2/ε4 genotype were excluded from all analyses since the APOEε2 allele seems to exert a protective effect on the risk of dementia and may counterbalance the effect of the APOEε4 allele.[32] Data analysis was performed using SPSS version 13.0 software (SPSS Inc, Chicago, Ill).

To test the associations of MMP-3 gene haplotypes with risk of dementia, AD, hippocampus volume and severity of WML, we used the program Haplo.Stats, which is implemented in the R software (http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html).[28-30] The probability for each haplotype pair in each individual was assigned and then an individual's phenotype was directly modeled as a function of each inferred haplotype pair, weighed by their estimated probability, to account for haplotype ambiguity. The haplo.score function of Haplo.Stats was used to test the associations.[30] We adjusted for age and sex and we computed global simulation P-values and simulation P-values for each haplotype. The number of simulations was set as 1000.

The associations of MMP-3 gene haplotypes with dementia, AD, hippocampus volume and WML was explored by using the haplo.glm function of Haplo.Stats, adjusting all models for age and sex.[29] This approach is based on a generalized linear model, and computes the regression of a trait on haplotypes and other covariates. In all analyses, the haplotype with the highest frequency was used as the reference category. We finally also repeated these analyses stratifying by APOEε4 genotype.

RESULTS

The genotypic distribution and demographic and clinical characteristics of the participants are shown in table 1. Genotype distributions for all tagging SNPs were in Hardy-Weinberg equilibrium.

There were 5,999 persons without dementia at baseline, with 44,992 person-years of follow-up (mean 7.5 person-years, SD 4.3 person-years). From these 5,999 persons, 610 (10.1%) subsequently developed dementia during follow-up. Out of those 610 persons who developed dementia, 468 (76.7%) were diagnosed with AD and 71 (11.6%) were diagnosed with vascular dementia (VaD).

In analyses relating the genotypes of the individual polymorphisms with the risk of incident dementia and AD, there was no association between carrier status of any polymorphism

Table 1. Baseline characteristics of the study sample in 5,999 persons followed prospectively

Women, n (%)	3532 (58.9)
Age, mean (SD), year	68.9 (8.7)
Educational level	
Low	2199 (36.7)
Intermediate	1592 (26.5)
High	2086 (34.8)
APOEε 4/- or 4/4 genotype, n (%)	1613 (26.9)
Diabetes mellitus, n (%)	609 (10.2)
Hypertension, n (%)	3573 (59.6)
Body mass index (kg/m^2), mean (SD)	26.3 (3.9)
Total cholesterol (mg/dl), mean (SD)	256.2 (47.0)
HDL (mg/dl), mean (SD)	51.9 (13.9)
Smoking, n (%)	3823 (63.7)
Intima media thickness (mm), mean (SD)	0.8 (1.6)
MMP3- 1187 (5A6A) genotype, n (%)	
5A5A	1595 (26.6)
5A6A	2933 (48.9)
6A6A	1471 (24.5)
MMP3- 2092 genotype, n (%)	
GG	257 (4.3)
GA	1838 (30.6)
AA	3769 (62.8)
MMP3- 59775 genotype, n (%)	
TT	4335 (72.3)
TA	1396 (23.3)
AA	98 (1.6)

HDL = high-density lipoprotein (HDL) cholesterol

Table 2. Relation between MMP3 polymorphisms and risk of incident dementia and AD in the Rotterdam Study

	Incident Dementia		Incident AD	
	No. (%) of incident dementia	**HR (95% CI)**	**No. (%) of incident AD**	**HR (95% CI)**
MMP3- 1187 (5A6A) genotype				
5A5A	163 (10.2)	reference	124 (8.0)	reference
5A6A	285 (9.7)	0.96 (0.79-1.16)	223 (7.8)	0.97 (0.78-1.21)
6A6A	157 (10.7)	1.11 (0.89-1.39)	121 (8.4)	1.11 (0.87-1.44)
p-value		0.4		0.4
MMP3- 2092 genotype				
AA	393 (10.1)	reference	299 (7.9)	reference
GA	201 (10.6)	1.06 (0.89-1.26)	163 (8.8)	1.10 (0.92-1.34)
GG	21 (7.9)	0.75 (0.48-1.16)	17 (6.5)	0.77 (0.47-1.26)
p-value		0.8		0.9
MMP3- 59775 genotype				
TT	459 (10.2)	reference	362 (8.2)	reference
TA	146 (10.2)	0.98 (0.81-1.18)	110 (7.9)	0.93 (0.75-1.15)
AA	7 (6.9)	0.65 (0.31-1.38)	6 (6.0)	0.74 (0.33-1.66)
p-value		0.2		0.3

Cox proportional hazards analyses. HR=hazard ratio, 95% CI= 95 percent confidence interval. All models are adjusted for age and sex.

Table 3. Difference in hippocampus volumes and severity of periventricular and subcortical white matter lesions between MMP3 polymorphism genotypes in the Rotterdam Scan Study (n=511)

	Number (%) of genotype	**Difference in hippocampus volume (95% CI)***	**Difference in severity of subcortical WML (95% CI) †**	**Difference in severity of periventricular WML (95% CI)****
MMP3- 1187 (5A6A) genotype				
5A5A	117 (24.8)	reference	reference	reference
5A6A	222 (47.1)	-0.25 (-0.44 - -0.06)	0.21 (-0.42-0.84)	0.12 (-0.29-0.55)
6A6A	132 (28.03)	0.04 (-0.17-2.44)	0.26 (-0.45-0.96)	0.34 (-0.13-0.81)
MMP3- 2092 genotype				
AA	292 (63.07)	reference	reference	reference
GA	140 (30.24)	-0.06 (-0.24-0.11)	0.21 (-0.37-0.78)	0.0003 (-0.38-0.38)
GG	31 (6.70)	0.18 (-0.13-0.50)	-0.12 (-1.19-0.94)	-0.47 (-1.17-0.23)
MMP3- 59775 genotype				
TT	330 (71.7)	reference	reference	reference
TA	122 (26.5)	-0.03 (-0.20-0.15)	0.15 (-0.44-0.75)	0.24 (-0.14-0.64)
AA	8 (1.7)	0.05 (-0.54-0.64)	0.68 (-1.44-2.80)	1.24 (-0.16-2.64)

* values are differences (95% CI) in volume (ml), adjusted for age and sex. † the total volume of subcortical WML was approximated based on number and size of lesions (range 0 to 30.0 mL). ** periventricular WML were graded semiquantitatively on a severity scale (0–3) at the frontal and occipital horns and the body of the lateral ventricle. The total periventricular WML score is the sum of these three scores.

Table 4. Relation between MMP3 haplotypes and dementia and AD in the Rotterdam Study

Haplotype	Dementia		AD	
	No. (%) of dementia	Model 1 OR (95% CI)	No. (%) of AD	Model 1 OR (95%CI)
Haplotype 1 (5A-A-T)	156 (10.2)	reference	118 (7.9)	reference
Haplotype 2 (6A-G-T)	20 (8.0)	1.0 (0.85-1.16)	16 (6.5)	1.0 (0.99-1.02)
Haplotype 3 (6A-A-T)	15 (10.9)	1.1 (0.95-1.36)	13 (9.6)	1.0 (0.99-1.02)
Haplotype 4 (6A-A-A)	7 (7.4)	0.9 (0.80-1.18)	6 (6.5)	1.0 (0.98-1.01)

All models are adjusted for age and sex.

Table 5. Relation between MMP3 haplotypes and hippocampus volume and severity of white matter lesions in the Rotterdam Scan Study

Haplotype	Difference in hippocampus volume ß (95% CI)*	Difference in severity of subcortical WML ß (95% CI) †	Difference in severity of periventricular WML ß (95% CI)**
Haplotype 1 (5A-A-T)	reference	reference	reference
Haplotype 2 (6A-G-T)	0.03 (-0.49-0.55)	0.15 (-0.66-0.96)	0.008 (-0.73-0.74)
Haplotype 3 (6A-A-T)	0.05 (-0.05-0.60)	0.15 (-0.66-0.96)	0.25 (-0.58-1.08)
Haplotype 4 (6A-A-A)	0.002 (-0.55-0.56)	0.05 (-0.76-0.86)	0.39 (-0.46-1.24)

* values are differences (95% CI) in volume (ml), adjusted for age and sex. † the total volume of subcortical WML was approximated based on number and size of lesions (range 0 to 30.0 mL). ** periventricular WML were graded semiquantitatively on a severity scale (0–3) at the frontal and occipital horns and the body of the lateral ventricle. The total periventricular WML score is the sum of these three scores.

and risk of dementia or AD (table 2). These results remained unchanged when carriers of the minor alleles were grouped together. In analyses relating the genotypes of the individual polymorphisms with hippocampus volume, carriers of the 5A allele had a smaller hippocampus volume compared with persons who were homozygeous for the 6A allele (table 3). However, this finding was non-significant after correction for multiple comparisons. There was no association between carrier status of any other polymorphism and hippocampus volume, and there was no association between individual polymorphisms and severity of periventricular or subcortical WML (table 3). There was no difference in these relations among strata of APOEε4 genotype.

Haplotypes were present in the following frequencies: haplotype 1 (5A-A-T): 51%, haplotype 2 (6A-G-T): 20%, haplotype 3= (6A-A-T): 15%, and haplotype 4 (6A-A-A): 14%. Since haplotype 1 was the most frequent haplotype, it served as reference category in further analyses.

In models relating these haplotypes with dementia and AD, there was no association between haplotypes and dementia or AD (Table 4). In analyses relating the haplotypes with hippocampus volume, there was no difference in hippocampus volume between haplotypes 2, 3, and 4 and haplotype 1 (table 5). In analyses relating the haplotypes with severity in periventricular and subcortical WML, there was no difference in WML severity between haplotypes

2, 3, and 4 and the reference haplotype (table 5). These relations also remained unchanged in strata of APOEε4 genotype.

DISCUSSION

In this study, there was no association between any individual polymorphism or MMP-3 haplotypes and risk and frequency of dementia and AD. In analyses relating the genotypes of the individual polymorphisms with hippocampus volume, carriers of the 5A allele had a smaller hippocampus volume compared with persons who were homozygeous for the 6A allele. However, this was a non-significant finding after adjustment for multiple comparisons, and in analyses relating MMP-3 haplotypes with hippocampus volume, there were no differences in hippocampus volume across haplotypes. There was no association between individual polymorphisms or haplotypes and severity of periventricular or subcortical WML. There were no differences of any of these relations between strata of APOEε4 genotype.

This study has important strengths. It was based on the population-based cohort of the Rotterdam Study with a large number of dementia cases and virtually complete follow-up with respect to dementia, and the population-based Rotterdam Scan Study with a large number of volumetric hippocampus assessments.

Several lines of evidence suggest a role of MMPs in the pathogenesis of AD. Findings of recent animal and post mortem studies suggest that MMP-3 is involved in the degradation of Aß.[1,2] There is also evidence for a reduced expression of MMP-3 in AD hippocampi,[3] suggesting that MMP-3 might play a role in selective neurodegeneration. Interestingly, MMP-3 is also one of the enzymes responsible for limited proteolysis of the hyaluronic acid-binding region of the versican-like aggregating proteoglycan, which results in aggregation of glial hyaluronic acid-binding protein (GHAP). This aggregation of GHAP has been found only in the white matter and in senile plaques of AD brain tissue.[33,34] MMP-3 also plays a role in the pathogenesis of atherosclerosis; common polymorphisms in the gene encoding MMP-3, in particular the 5A6A promotor polymorphism, have been repeatedly associated with the risk of coronary heart disease, myocardial infarction, and atherosclerosis,[4-8] which in turn have been related to the risk of AD in several studies.[9-11]

MMP-3 also plays a central role through activation of latent-type MMPs such as MMPs -1, -8, -9, and -13, from which some are involved in the degradation of Aß.[1,2] MMP-9 (Gelatinase B) furthermore cleaves the LEU34-Met35 chemical bond within the transmembrane domain of the Aß peptide.[1,35] Also, increased plasma levels of MMP-9 have been observed in persons with AD compared with non-demented persons of the same age, and MMP-9 protein overexpression was confirmed in AD tissue compared with age-matched control tissue.[1,35]

Only three previous studies explored the association between variation in genes encoding MMPs and dementia in a population-based observational setting.[12-14] All assessed the impact of individual single nucleotid polymorphisms (SNPs) on the frequency of dementia without taking the complete variation in the MMP-3 gene into account. Helbecque et al. reported in a first study a weak effect of the MMP-9 1562C>T high activity allele on the risk of AD in APOEε4 non-carriers in a European population.[13] In a second, very recently published study, they reported an association of the 6A-allele of the 5A6A promotor polymorphism with an increased risk of dementia in persons without the APOEε4 allele.[14] Shibata et al.[12] explored the association of four common polymorphisms in each the MMP-3 and MMP-9 genes (rs3918248, rs2664538, rs2250889, rs2274756, MMP-3 5A6A promotor polymorphism, rs3025079, rs520540, and rs679620) with sporadic AD in a population of Japanese descent. In their study, there was no association between any of the individually examined SNPs and risk of AD.

To our knowledge, the present study is the first population-based study evaluating the association between the comprehensive variation in the MMP-3 gene and risk of dementia and AD and pathological changes potentially underlying these disorders. Consistent with the findings by Shibata et al.,[12] we did not find an association between genetic variation in the MMP-3 gene and risk of dementia, AD or severity of WML in overall analyses or strata of APOEε4 genotype. We did observe an association between the 5A allele of the 5A6A promotor polymorphism and a decreased hippocampus volume. This finding, however, was not significant after adjustment for multiple testing, and was not replicated in analyses using haplotypes representing the comprehensive common variation in the MMP-3 gene.

Helbecque et al.reported an association of the 6A-allele of the 5A6A promotor polymorphism with an increased risk of dementia in APOEε4 non-carriers based on analyses relating the 5A6A promotor polymorphism with overall dementia, AD and non-AD in two separate case-control samples, and then in pooled analyses.[14] All analyses were initially performed in the overall samples and subsequently in strata of APOEε4 genotype. The conclusion of a role of MMP-3 in dementia etiology was drawn from the observation that the 6A-allele of the 5A6A promotor polymorphism was associated with an increased risk of dementia in APOEε4 non-carriers in the first case-control study and the pooled analysis. The p-values of these analyses were after adjustment for multiple testing borderline-significant. There was no association between the 6A allele and dementia risk in the second case-control study, and there was no association between the 6A allele and subtypes of dementia in any of the three studies performed. Summarizing the findings of all analyses in the study, these results do not compellingly support the hypothesis that MMP-3 is causally involved in dementia aetiology, and may therefore be in agreement with our findings.

Although MMP-3 expression is regulated primarily at the level of transcription, there remains the possibility that relations between MMP plasma levels and dementia observed in previous studies might not be causal but caused by residual confounding. Analyses relating variation in the MMP-3 gene with disease outcome overcome this problem. Alleles of the MMP-3 gene influencing levels of MMP-3 and latent-type MMPs, are transmitted from parent to offspring at random, and thus factors that may confound associations between MMP levels and dementia are likely to be evenly distributed among those with and without alleles causing high MMP-3 levels (Mendelian Randomization).[36]

In summary, our study does not suggest that MMP-3 is causally involved in the pathogenesis of dementia or AD, and that the APOEε4 allele modifies this association. More studies are needed to gain insight into the exact mechanisms underlying the associations between MMPs and AD pathology observed in animal and post mortem studies.

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4

Stroke and cognitive decline

4.1

Stroke and memory performance in elderly without dementia

ABSTRACT

Background. There is conflicting data showing that stroke is associated with a higher risk of dementia and a more severe decline in persons with cognitive impairment. However, if cerebrovascular disease is directly related to cognitive decline in the absence of cognitive impairment or dementia remains unclear. **Objective.** To examine the association between stroke and changes in cognitive function over time in elderly persons without dementia at baseline. **Design.** The results of neuropsychological tests from several intervals over a five-year-period were clustered into domains of memory, abstract/visuospatial and language in 1271 elderly without dementia or cognitive decline. Stroke was related to the slope of performance in each cognitive domain using generalized estimating equations. **Results.** Memory performance declined over time while abstract/visuospatial and language performance remained stable over the study period. Stroke was associated with a more rapid decline in memory performance, while there was no association between stroke and decline in abstract/visuospatial or language performance. The association between stroke and decline in memory performance was strongest for men and for persons without an APOEε4 allele. A significant association between stroke and decline in abstract/visuospatial performance was also observed for persons without the APOEε4 allele. **Conclusion.** A history of stroke is related to a progressive decline in memory and abstract/visuospatial performance especially among men and those without an APOEε4 allele.

INTRODUCTION

Cerebrovascular disease and dementia are among the most common diseases in aging societies. According to the WHO, cerebrovascular disease is the second leading cause of mortality in western societies and the major cause of long-term disability leaving 30% disabled.[1] About 1 percent of people aged 65-69 years have dementia, and this proportion increases with age to approximately 60% percent for people over the age of 95.[2]

The role of stroke in the pathogenesis of cognitive decline remains unclear. Longitudinal population-based studies indicate that vascular risk factors, such as diabetes or hypertension are associated with stroke, which in turn may be related to the development of vascular dementia and Alzheimer's disease (AD).[3,4] We previously reported a relation between stroke and the risk of AD.[5] Vascular risk factors have also been associated with mild cognitive impairment (MCI),[3] and there is evidence that cerebrovascular disease is associated with more progressive decline in persons with cognitive impairment.[6,7] However, whether or not cerebrovascular disease is directly related to cognitive decline in the absence of cognitive impairment or dementia remains unclear.

The objective of this study was to determine if the effects of stroke result in a decline in memory and other cognitive functions in elderly persons who do not have cognitive impairment or dementia.

METHODS

Subjects and Setting. Participants were part of a longitudinal study of Medicare recipients, aged 65 years or older, residing in northern Manhattan (Washington Heights, Hamilton Heights, Inwood).[8] Each participant underwent an in-person interview of general health and function at the time of study entry followed by a standard assessment, including medical history, physical and neurological examination as well as a neuropsychological battery.[9] Baseline data were collected from 1992 through 1994. Follow-up data were collected during evaluations at sequential intervals of approximately 18 months, performed from 1994 to 1996, 1996 to 1997, and 1997 to 1999. In this elderly population, some participants did not complete follow up at all intervals due to refusal to participate further, relocation or death. About one half of participants were evaluated at the third follow-up visit. This study was approved by the institutional review board of the Columbia-Presbyterian Medical Center.

The participants selected for this study were without dementia or cognitive impairment at baseline, complete stroke information, and with at least 3 follow-up intervals.

Of the 2126 individuals who underwent clinical assessment at baseline, 346 (16.3%) individuals were excluded because they were demented at the initial intake examination. In-

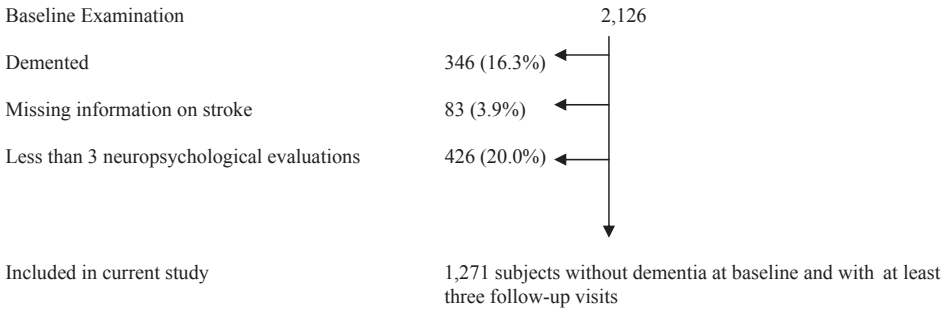

Figure 1. Description of sample size

formation on stroke was unavailable in 83 (3.9%) cases and 426 (20.0%) subjects had less than three follow-up visits with neuropsychological evaluation (Figure 1). Thus, the study focused on 1271 individuals without dementia or cognitive impairment at baseline, followed over a 5-year interval.

Clinical assessments. Data included medical, neurological, and neuropsychological evaluations.[9,10] All participants underwent a standardized neuropsychological test battery in either English or Spanish.[9] Orientation was evaluated using parts of the modified Mini-Mental State Examination.[11] Language was assessed using the Boston Naming Test,[12] the Controlled Word Association Test,[13] category naming, and the Complex Ideational Material and Phrase Repetition subtests from the Boston Diagnostic Aphasia Evaluation.[12] Abstract Reasoning was evaluated using WAIS-R Similarities subtest,[14] and the non-verbal Identities and Oddities subtest of the Mattis Dementia Rating Scale.[15] Visuospatial ability was examined using the Rosen Drawing Test,[16] and a matching version of the Benton Visual Retention Test.[17] Memory was evaluated using the multiple choice version of the Benton Visual Retention Test[17] and the seven subtests of the Selective Reminding Test:[18] total recall, long-term recall, long-term storage, continuous long-term storage, words recalled on last trial, delayed recall, and delayed recognition. This neuropsychological test battery has established norms for the same community.[19]

Definition of dementia and cognitive impairment. Results from the neurological, psychiatric and neuropsychological examinations were reviewed in a consensus conference comprised of physicians, neurologists, neuropsychologists and psychiatrists. Based on this review all participants were assigned to one of three categories: normal cognitive function, cognitive impairment without dementia, or dementia. Cognitive impairment without dementia was defined as the presence of abnormal neuropsychological tests for age, sex, and education group without significant cognitive impairment, and a Clinical Dementia Rating (CDR) of 0.5.[20] Dementia was defined as the presence of abnormalities in several cognitive domains in neuropsychiatric testing accompanied by significant functional impairment (CDR ≥ 1).

Stroke. Stroke was defined according to the WHO criteria.[21] At baseline, the presence of stroke was ascertained from an interview with participants and their informants. Positive response(s) to any 1 of the 8 questions shown in Figure 2 was considered as suggestive of a history of stroke. Persons with stroke were confirmed through their medical records, 85% of which included results of brain imaging. The remainder were confirmed by direct examination.

1. Have you ever had a stroke of the brain, ministroke, CVA (cerebrovasular accident), or TIA (transient ischemic attack)?
2. Did a doctor tell you that you had a stroke of the brain, ministroke, CVA (cerebrovascular accident) or TIA (transient ischemic attack)?
3. Did you have a stroke of the brain, ministroke, CVA (cerebrovascular accident) or TIA (transient ischemic attack) within the past year?
4. Have you ever had a sudden paralysis (weakness) or numbness (loss of sensation) on one side of the body but not the other?
5. Have you ever suddenly lost the use of speech (not being able to talk at all) or suddenly had slurred speech (not being able to say words clearly)?
6. Have you ever had sudden loss of consciousness with severe headache, nausea, vomiting?
7. Did the stroke or symptoms last more than 24 hours?
8. Have the stroke symptoms continued without ever going away?

Figure 2. Survey questions assessing stroke. Stroke was defined as an affirmative answer to one of these questions.

APOE Genotyping. APOE genotypes were determined as described by Hixson and Vernier[22] with slight modification.[23] We classified persons as homozygeous or heterozygeous for the APOE ε4 allele or not having any ε4 allele.

Other covariates. Diabetes mellitus and hypertension were defined by self-report at baseline and at each follow-up interval or by the use of disease specific medications. Blood pressure measurements were also considered in the definition of hypertension. Heart disease was defined as a history of myocardial infarction, congestive heart failure or angina pectoris at any time during life. Body mass index (BMI) was calculated by the formula BMI = weight (Kg)/ height $(m)^2$. Smoking was assessed by self-report and categorized as never, past and current smoking.

Statistical Methods. A factor analysis was performed using data from the entire cohort with the 15 neuropsychological measures using a principal component analysis with varimax rotation and Kaiser normalization.[24] This analysis resulted in three factors: 1) a memory factor, in which the seven subtests of the Selective Reminding Test[18] were the main contributors; 2) a abstract/visuospatial factor, where visuospatial and tests of reasoning were the main contributors; and 3) a language factor, in which language measures from the Boston Naming Test,[12] Controlled Oral Word Association Test,[13] and the WAIS-R Similarities[14] were the main contributors. We calculated cognitive scores for each participant at each visit by adding the scores of the measures that contributed most to each factor (tests with correlations of 0.5 or higher). Each factor score was normally distributed.

Generalized estimating equations (GEE)[25] were used to examine changes in each cognitive domain over time. The dependent variables were the factor scores, and the independent variables were stroke, time (included as a continuous variable, and representing the time of follow-up of each participant), and the interaction of stroke and time. After adjusting for age and gender, subsequent models were adjusted for age, gender, education, ethnic group, APOEε4 genotype, BMI, hypertension, heart disease, diabetes and smoking. In these full models age, education and BMI were included as continuous variables, ethnic group, APOEε4 genotype and smoking as multilevel categorical variables, and hypertension, heart disease and diabetes as dichotomized (not present vs. present) variables.

The GEE analysis yielded coefficient values that represent the associations between a factor score and variables included in the model. There were three main coefficients of interest in each model: one comparing the stroke groups (stroke yes/no) at baseline, one relating the change in cognitive scores with time, and an interaction term for stroke and time. A significant p value for the coefficient comparing stroke groups at baseline indicates a difference between two groups at baseline. A significant p value for the coefficient of time indicates a statistically significant change in a cognitive score over the total duration of follow-up. A significant p value for the interaction coefficient indicates a difference in the rate of change in a factor score depending on the stroke group; this is the main variable of interest for the interpretation of the analyses. All analyses were repeated after stratifying for gender and APOEε4genotype.

RESULTS

The mean age of the sample was 76.2 ± 6.0 years, 69.6% were women, 45.1% were Hispanic, 20.6% were White, and 33.7% were Blacks. The mean of years of education was 8.6 ± 4.6, and 20.8% were homozygous or heterozygous for the APOE-ε4 allele. The mean BMI was 27.1 ± 5.1, and 29.8% of the subjects reported having diabetes, 55.1% hypertension and 29.5% heart disease. 7.6% had a history of stroke. Persons with stroke at baseline had a higher prevalence of diabetes and hypertension than persons without stroke (Table 1). There were no significant differences in stroke prevalence among gender or ethnic groups.

In the GEE analysis memory declined significantly over time (ß=-1.6, p=0.005), while abstract/visuospatial and language performance remained stable over the study period (Table 2). A history of stroke was associated with more rapid decline in memory performance over time (ß = -3.6, p for interaction of stroke and time = 0.04). There was no relation between stroke and decline in abstract/visuospatial (ß =-0.1, p for interaction of stroke and time = 0.9) or language performance (ß =0.1, p for interaction of stroke and time = 0.5). There was also no relation when analyses were repeated for individual tests in the abstract/visuospatial and language domains.

Table 1. Comparison of demographic characteristics between persons with and without stroke at baseline

Covariates	No Stroke (n=1174)	Stroke (n=97)
Men	359 (30.6)	27 (27.8)
Women	815 (69.4)	70 (72.2)
Education, mean (SD), year	8.6 (4.6)	8.9 (4.3)
Age, mean (SD), year	76.2 (6.0)	76.3 (5.9)
Body mass index, mean (SD)	27.1 (5.1)	27.3 (4.6)
Ethnic group ‡		
White/Non-Hispanic	239 (20.4)	23 (23.7)
Black/Non-Hispanic	390 (33.2)	38 (39.2)
Hispanic	538 (45.8)	35 (36.1)
APOE genotype 4/4	21 (2.2)	--
APOE genotype 4/-	255 (26.2)	19 (26.0)
APOE genotype -/-	699 (71.7)	54 (74.0)
Diabetes	352 (29.9)	30 (35.4)*
Heart disease	343 (29.2)	28 (29.1)
Hypertension	630 (53.9)	71 (75.5)*

Values are expressed as number (percentage) unless otherwise indicated. Some percentages are based on an incomplete sample due to small amounts of missing data. ‡ Classified by self-report using the format of the 1990 US census.[49] *significant at a 0.05 level vs. group without stroke.

All analyses were repeated stratifying by gender and APOEε4 genotype. While in both men and women as well as APOE ε4 carriers and non-carriers memory performance significantly declined over time, the association between stroke and decline in memory performance over time (stroke*time interaction) was stronger in men (ß=-10.1, p=0.005, Table 3; p for interaction gender*stroke*time = 0.07) and persons without APOEε4 allele (ß =-4.1**,** p=0.07, Table 4; p for interaction APOEε4*stroke*time=0.09). Persons without APOEε4 allele also showed a significant stroke*time interaction indicating that abstract/visuospatial function declined faster among APOEε4-non-carriers (ß=-1.1, p=0.04; p for interaction APOEε4*stroke*time= 0.07). Thus, memory and abstract/visuospatial function declined at a faster rate in men or persons who lacked the APOEε4 allele with stroke compared to women or APOEε4 carriers. These associations remained unchanged after adjusting for age, education, ethnic group, BMI, hypertension, heart disease, diabetes and smoking. There was no association between stroke and language performance.

COMMENT

In this study the performance in memory, abstract/visuospatial and language domains declined over time in individuals free of dementia or cognitive impairment at baseline. A history

Table 2. Relationship of stroke and time of follow-up to memory, abstract/visuospatial and language performance in 1271 healthy elderly over 7 years

	Model 1		Model 2	
Variable	**Estimated ß (SE)**	**p-value**	**Estimated ß (SE)**	**p-value**
Memory Performance				
Time	-1.6 (0.6)	0.005	-1.6 (0.6)	0.006
Stroke	-2.3 (5.9)	0.7	-1.2 (6.2)	0.8
Time*Stroke	-3.6 (1.8)	0.04	-3.5 (1.9)	0.05
Abstract/visuospatial Performance				
Time	0.1 (0.3)	0.7	0.1 (0.3)	0.6
Stroke	-2.2 (2.8)	0.4	0.3 (3.1)	0.9
Time*Stroke	-0.1 (0.6)	0.9	-0.2 (0.6)	0.7
Language Performance				
Time	-0.1 (0.1)	0.9	0.1 (0.1)	0.9
Stroke	-0.1 (0.5)	0.8	-0.1 (0.5)	0.8
Time*Stroke	0.1 (0.1)	0.5	0.1 (0.1)	0.4

Model 1 is adjusted for age and gender, Model 2 is adjusted for age, gender, education, ethnic group, APOEε4 genotype, BMI, hypertension, heart disease, diabetes and smoking.

of stroke was associated with faster decline only in memory performance. When stratified by sex or APOEε4 genotype, stroke was associated with a faster decline in memory or abstract/visuospatial performance in men or persons lacking the APOEε4 allele.

The mechanisms by which stroke increases the risk of cognitive decline are not clear. Stroke could increase the risk of cognitive decline by destruction of brain parenchyma and atrophy such as in the case of vascular dementia or AD associated with stroke,[26,27] or by causing damage in strategic locations that lead to amnestic syndromes, such as thalamic strokes.[28,29] Stroke could also increase the risk of cognitive decline by increasing the deposition of amyloid β, the key step in the pathogenesis of Alzheimer's disease,[30,31] or by a combination of these different mechanisms. It is also possible that the occurrence of stroke adds cognitive deficits in persons with subclinical AD that bring them over the diagnostic threshold, without directly affecting the deposition of amyloid beta, and that stroke does not have a direct specific effect on AD.

Studies examining the role of stroke in cognitive function reported inconsistent results. The Framingham Study reported in a nested case-control study a doubled risk of dementia after baseline stroke,[32] and a similar observation has been made earlier by a longitudinal study assessing the risk of incident dementia after cerebral infarction in 971 subjects in Minnesota.[33]

Table 3. Relationship of stroke and time of follow-up to memory, abstract/visuospatial and language performance in 1271 elderly persons over 7 years of follow-up stratified by gender

	Model 1		Model 2	
Variable	**Estimated ß (SE)**	**p-value**	**Estimated ß (SE)**	**p-value**
Memory Performance				
Men				
Time	-1.8 (1.1)	0.1	-3.2 (1.2)	0.009
Stroke	8.6 (13.3)	0.5	12.0 (15.8)	0.5
Time*Stroke	-10.1 (3.6)	0.005	-9.9 (3.7)	0.008
Women				
Time	-1.5 (0.7)	0.02	-1.6 (0.8)	0.04
Stroke	-6.7 (6.1)	0.2	-2.4 (7.6)	0.8
Time*Stroke	-1.3 (1.8)	0.4	-0.7 (2.1)	0.7
Abstract/visuospatial Performance				
Men				
Time	0.6 (0.5)	0.2	0.4 (0.5)	0.4
Stroke	3.6 (4.9)	0.4	8.6 (4.6)	0.07
Time*Stroke	-0.8 (1.2)	0.5	-0.9 (1.4)	0.5
Women				
Time	-0.2 (0.3)	0.6	-0.1 (0.3)	0.8
Stroke	-4.7 (3.3)	0.2	-1.7 (4.39)	0.7
Time*Stroke	0.2 (0.6)	0.8	0.2 (0.6)	0.8
Language Performance				
Men				
Time	-0.1 (0.1)	0.5	-0.1 (0.1)	0.4
Stroke	0.5 (0.8)	0.5	0.8 (0.9)	0.4
Time*Stroke	0.3 (0.2)	0.2	0.2 (0.2)	0.4
Women				
Time	0.1 (0.1)	0.7	0.1 (0.1)	0.8
Stroke	-0.4 (0.6)	0.5	0.2 (0.7)	0.8
Time*Stroke	0.1 (0.1)	0.9	0.1 (0.2)	0.7

Model 1 is adjusted for age, Model 2 is adjusted for age, education, ethnic group, APOEε4 genotype, BMI, hypertension, heart disease, diabetes and smoking

Table 4. Relationship of stroke and time of follow-up to memory, abstract/visuospatial and language performance in 1271 elderly persons over 7 years of follow-up stratified by APOE genotype

	Model 1		Model 2	
Variable	**Estimated ß (SE)**	**p-value**	**Estimated ß (SE)**	**p-value**
Memory Performance				
-/- APOEε4 genotype				
Time	-1.3 (0.7)	0.06	-1.7 (0.8)	0.04
Stroke	-1.5 (7.3)	0.8	3.4 (8.4)	0.7
Time*Stroke	-4.1 (2.2)	0.07	-4.2 (2.4)	0.09
-/4 or 4/4 APOEε4 genotype				
Time	-2.5 (1.1)	0.02	-3.2 (1.2)	0.008
Stroke	-3.4 (10.8)	0.8	-1.3 (14.0)	0.9
Time*Stroke	-2.6 (2.9)	0.4	-1.4 (3.2)	0.6
Abstract/visuospatial Performance				
-/- APOEε4 genotype				
Time	1.0 (3.5)	0.8	3.8 (4.1)	0.4
Stroke	-0.1 (3.1)	0.9	-0.1 (0.3)	0.9
Time*Stroke	-1.1 (0.5)	0.04	-1.0 (0.6)	0.06
-/4 or 4/4 APOEε4 genotype				
Time	0.4 (0.4)	0.4	0.4 (0.4)	0.4
Stroke	-6.7 (4.7)	0.1	-3.3 (6.0)	0.6
Time*Stroke	1.7 (1.1)	0.1	2.0 (1.0)	0.07
Language Performance				
-/- APOEε4 genotype				
Time	0.1 (0.1)	0.8	-0.1 (0.1)	0.9
Stroke	-0.1 (0.6)	0.9	0.3 (0.6)	0.6
Time*Stroke	0.1 (0.1)	0.2	0.1 (0.1)	0.2
-/4 or 4/4 APOEε4 genotype				
Time	-0.1 (0.1)	0.8	-0.1 (0.1)	0.8
Stroke	-0.4 (0.8)	0.6	0.1 (0.8)	0.9
Time*Stroke	-0.1 (0.2)	0.8	-0.1 (0.3)	0.7

Model 1 is adjusted for age and gender, Model 2 is adjusted for age, gender, education, ethnic group, BMI, hypertension, heart disease, diabetes and smoking

Hospital-based cohorts with a follow-up shorter than 3 months also observed an increased risk of incident dementia after stroke,[34-36] and we previously reported an increased risk of dementia after stroke.[37] Others have not found an association between cerebrovascular disease and cognitive impairment or dementia.[38,39]

Our results are consistent with studies showing an increased risk of AD in persons with stroke.[5,40] The main cognitive domain affected in AD is memory[41,42] and it seems reasonable to postulate that if stroke is related to a higher risk of AD, it must be related to decline in memory. Furthermore, it seems that this effect is independent of APOE genotype, which is in agreement with studies indicating an increased risk of AD with stroke in persons without the APOEε4 allele.[43]

Stroke has been found to be related to impairment in frontal executive functions.[44-46] The domain in our study that better represents this construct is abstract/visuospatial performance, and we found no association of stroke to differences in this domain at baseline or with follow-up. The reasons for this negative finding may be that our cognitive battery lacked better measures of frontal/executive functions, such as the Color trails.[47]

There are several potential alternative explanations for our findings. One is chance, particularly in the context of multiple comparisons. However, this study was based on our previous findings relating stroke to a higher risk of AD.[5] Also, this study is consistent with other studies as described in the previous paragraph; these facts make chance due to multiple comparisons an unlikely explanation for our findings.[48] One of our findings was that stroke was related to faster cognitive decline in men. The strata for men was much smaller than for women, and only 27 men had stroke, and this could also result in chance findings. These findings should be reproduced in a larger cohort. Another potential explanation is bias. For example, that only subjects with preclinical AD reported stroke while subjects that would not develop AD did not. This type of reporting bias seems unlikely and we excluded cases of prevalent dementia or cognitive impairment that could have influenced our results. Further, if lower education is related to stroke, and persons with lower education are more likely to be diagnosed with AD, then it is possible that a relation between stroke and cognitive decline could be confounded by socioeconomic status. We adjusted for years of education and ethnicity as markers of socioeconomic status to account for this possibility. However, it is possible that stroke is related to other behaviors related to poor health, that in turn may increase the risk of AD, that we could not adjust for, and we cannot eliminate the possibility of lack of control for unknown confounders as a potential explanation for our findings. Finally, a potentially major source of bias, and the main limitation of our study, is the lack of ascertainment of sub-clinical cerebrovascular disease in persons without stroke. We also lacked information on the location and severity of cerebrovascular disease. If sub-clinical stroke is associated with cognitive decline as we hypothesized for clinical stroke, then our results are biased toward the null. Thus, our

findings seem to underestimate the true relation of stroke to memory decline, and our negative findings for language and visuospatial abilities may be explained by this source of bias.

This study has several strengths. We had a comprehensive and sensitive neuropsychological battery validated for use in the communities of northern Manhattan.[9] We also excluded from our analyses persons with dementia and cognitive impairment without dementia at baseline that may have biased the analyses, and had several evaluation time points that allowed prospective analyses.

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4.2

Pre-stroke Cognitive Performance, Incident Stroke and Risk of Dementia

ABSTRACT

Background and Objective. Several studies indicate that stroke increases the risk of dementia. Most of these studies, however, used prevalent information of stroke or were performed in clinical settings, lacking the ability to take accurately assessed pre-stroke cognitive function into account. We explored in a prospective cohort study whether first ever incident stroke is related to a higher risk of subsequent dementia or AD, and whether this association is dependent on pre-stroke level of cognitive function. We also assessed the effect of pre-stroke measures of other common risk factors for cognitive decline on the risk of post-stroke dementia and AD. **Methods and Design.** Prospective population-based cohort study. Cox proportional hazard models were used to relate incident stroke as a time-varying exposure with the risk of dementia and AD in 6724 participants of the Rotterdam Study without dementia or stroke at baseline. Subsequently Cox-proportional hazard models were performed to assess whether this association is dependent on pre-stroke measures of cognitive performance and other common risk factors for cognitive decline. **Results.** Incident stroke was associated with a more than doubled risk of subsequent dementia, independent of pre-stroke cognitive performance and other potential risk factors for cognitive decline (HR 2.1, 95% CI 1.55-2.81). When the analyses were restricted to vascular dementia (VaD) only as the outcome, the risk was four-fold increased (HR 4.0, 95% CI 2.82-6.19).When the analyses were restricted to AD only as the outcome, the association between incident stroke and the risk of subsequent AD was attenuated (HR 1.3, 95% CI 0.87-1.98). **Conclusion.** Stroke increases the risk of subsequent dementia independent from pre-stroke level of cognitive function, or pre-stroke measures of other potential risk factors for cognitive decline. By definition, the association of stroke was stronger with vascular dementia syndromes than with Alzheimer's disease.

INTRODUCTION

Cerebrovascular disease and dementia are among the most common diseases in aging societies. According to the WHO, cerebrovascular disease is the second leading cause of mortality in western societies and the major cause of long-term disability leaving 30% disabled.[1] About 1 percent of people aged 65-69 years have dementia, and this proportion increases with age to approximately 60% percent for people over the age of 95.[2]

Epidemiologic evidence is accumulating that both disorders are linked. In their recently published review, Leys et al.[3] summarized the previous studies that explored the impact of stroke on the risk of post-stroke dementia (PSD). According to these studies, stroke considerably increases the risk of dementia, with prevalence rates ranging from 13.6 to 32% within 3 months to 1 year after stroke, and incidence rates of new onset dementia after stroke ranging from 24% within 3 years to 33.3% within 5 years.[3-13] The subtype of PSD differs among the studies depending on mean age of patients, ethnicity, diagnostic criteria used, and time after stroke. In the studies performed in western societies, the proportion of patients with presumed post-stroke Alzheimer's disease was reported to be between 19% and 61%.[4,5,11,13-17]

Patient-related demographic and clinical determinants of PSD reportedly include increasing age, low educational level, dependency before stroke, various vascular risk factors, measures of structural and functional heart disease, epileptic seizures, and cerebral small-vessel disease.[6-9,11-13,15-23] As stroke-related determinants of PSD, have been observed cause and location of stroke, stroke severity, and stroke recurrence.[5,13,14,16,24-26] While age has been consistently considered a risk factor for PSD,[5,6,9,11-14] data concerning the effect of education, sex, vascular risk factors, previous stroke, or structural brain changes remained controversial among the studies.[5,8-20,22,25-27]

To accurately interpret the impact of stroke on the risk of PSD, pre-stroke level of cognitive function has to be taken into account. As stated by Leys et al,[3] the studies that related pre-stroke cognitive performance with PSD reported a higher risk of PSD after 3 months[6,9,10,13] and 3 years[8,14] in persons with pre-stroke cognitive decline compared with persons without cognitive impairment before stroke. These studies, however, had been obtained from stroke cohorts assessing pre-stroke cognitive function either by measuring cognitive performance at time of hospital admission, or by using dementia diagnoses based on pre-stroke medical records.[7-9,11,13] Accurate assessment of the association between stroke and the risk of PSD taking pre-stroke cognitive performance into account, requires, however, assessment of pre-stroke cognitive status using an adequate neuropsychogical test battery, a long enough-follow-up time between pre-stroke cognitive assessment and occurrence of stroke, and subsequently a long enough follow-up time between the incident stroke and subsequent dementia or censoring.

The objective of the present study was to elucidate the true impact of stroke on the risk of PSD as a function of pre-stroke cognitive performance by assessing the impact of pre-stroke cognitive performance on the association between incident stroke and risk of subsequent dementia and AD in the large prospective population-based Rotterdam Study. We also sought to assess the effect of pre-stroke measures of other common risk factors for cognitive decline on the risk of dementia and AD after stroke.

METHODS

Participants and Setting. The Rotterdam Study is a population-based prospective cohort study that was designed to investigate the incidence and causes of cardiovascular, neurodegenerative, locomotor, and ophthalmologic diseases in the elderly.[28] From 1990 to 1993, all 10 275 residents aged ≥55 years of Ommoord, a district of the city of Rotterdam, were invited to participate, and 7983 (78%) men and women agreed. The Medical Ethics Committee of the Erasmus Medical Center approved the study, and written informed consent was obtained from all participants. During the baseline examination (1990-1993), a research assistant interviewed participants in their homes and obtained information on current and past health, medication, lifestyle, and risk factors for chronic diseases. In addition, participants visited the research center twice for baseline clinical examinations. Follow-up examinations took place in 1993-1994,1997-1999 and 2002-2004. Through linkage with records of general practitioners, the entire cohort was continuously monitored for morbidity and mortality. This follow-up information was available for all participants until January 1, 2005.

From the 7983 participants who underwent baseline examination, 7528 were screened for dementia (94.3%). From these, 482 persons (6.4%) were diagnosed with prevalent dementia, 175 persons (2.2%) had at baseline a history of stroke, and 147 persons (2.0%) did not agree to give informed consent for collecting stroke information. The final analytic sample included in this study comprised 6724 persons without dementia or stroke at baseline. Follow-up with the respect to dementia and stroke was nearly complete (96.7%).

Diagnosis of Dementia and Alzheimer Disease. Diagnostic procedures for dementia and Alzheimer disease have been described in detail.[29] At baseline and both follow-up examinations, a three-stage protocol was used to screen all participants cognitively with the Mini-Mental State Examination (MMSE)[30] and the Geriatric Mental State schedule (GMS) organic level.[31] If subjects scored lower than 26 on the MMSE or higher than 0 on the GMS organic level, the Cambridge Examination of Mental Disorders in the Elderly (CAMDEX)[32] was administered. The CAMDEX also included an informant interview. Finally, participants in whom dementia was suspected were examined by a neurologist and neuropsychologist and, if possible, underwent

magnetic resonance imaging of the brain. In addition, the total cohort was continuously monitored for incident dementia cases through computerized linkage between the study database and computerized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.[29] The diagnoses of dementia and Alzheimer disease were based on *Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition* (*DSM-III-R*) criteria[33] and the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria,[34] respectively, and were made by a panel of a neurologist, neuropsychologist, and research physicians who reviewed all existing information.[29]

Assessment of stroke. History of stroke at time of enrollment into the Rotterdam Study was assessed by the question 'did you ever suffer from a stroke, diagnosed by a physician?' Positive answers to this question were verified by review of medical records. After baseline assessment participants were continuously monitored for major events through automated linkage of the study database with files from general practitioners and the municipality. In addition, nursery home physicians' files and files from general practitioners of participants who moved out of the district were scrutinized. For reported events, additional information including brain imaging was obtained from hospital records. Research physicians discussed information on all potential strokes and transient ischemic attacks with an experienced stroke neurologist to verify all diagnosis. Subarachnoid hemorrhages and retinal strokes were excluded from the stroke diagnosis. Then strokes were subclassified into hemorrhagic or ischemic stroke based on neuroimaging. Strokes which could not be subclassified as ischemic or hemorrhagic, were called unspecified.

Assessment of other covariates. At baseline, trained investigators interviewed all participants at home, collecting information on socioeconomic status, current health status and medical history. In addition, clinical measures were obtained at the research center. Level of education was categorized into 3 groups: low (primary education only); intermediate (lower vocational or general education); and high (intermediate or higher vocational or general education, college, or university). Smoking habits were categorized as ever smoking and non-smoking. Body mass index was calculated using the formula [weight (kg)/length (m^2)]. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. Diabetes mellitus was defined as a random or postload glucose level ≥11.1 mmol/L or a history of diabetes or the use of blood glucose-lowering medication.

Nonfasting blood samples were drawn and immediately frozen. Total cholesterol, high-density lipoprotein cholesterol, and glucose were measured within 2 weeks, as described previously.[35] Levels of serum c-reactive protein (CRP) were determined by the rate near infrared particle immunoassay method (Immage high-sensitivity CRP, Beckman Coulter).

Furthermore, ultrasonography of both carotid arteries was performed. As an indicator of atherosclerosis of the carotid arteries, we used intima media thickness (IMT). Common carotid IMT was determined as the average of the maximum IMT of near- and far-wall measurements, and the average of left and right common carotid IMT was computed.[36] Apolipoprotein E (APOE) genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis.[37] We dichotomized APOE genotype into presence or absence of the apolipoprotein E*4 (APOE*4) allele. APOEε2ε4 carriers were excluded from the analyses.

Statistical Methods. First we evaluated the demographic and clinical characteristics of the study sample at baseline. Then we performed Kaplan-Meier analyses to determine the proportion of participants surviving free of dementia among persons without incident stroke, persons with incident stroke with *normal* pre-stroke cognitive function (last MMSE score before stroke ≥ 25), and persons with incident stroke with *low* pre-stroke cognitive function (last MMSE score before stroke <25). In these analyses, the date of onset of dementia was considered to be the date of the visit at which dementia was diagnosed.

Then we performed Cox proportional hazards analyses relating incident stroke as a time-varying exposure with the risks of subsequent incident dementia, VaD and AD. We initially adjusted all models for sex and age, subsequently we adjusted for sex, age, APOEε4 genotype and education in later analyses. To explore the impact of pre-stroke cognitive function on the association between incident stroke and subsequent dementia, VaD or AD, we then repeated all analyses adding an interaction term to the model that contained variables for incident stroke (yes/no) and pre-stroke cognitive function. In these analyses, pre-stroke cognitive function was first assessed using baseline measures of MMSE, and then using rate of decline in MMSE over time before occurrence of stroke or censoring.

To explore the effect modification of the association between incident stroke and subsequent incident dementia, VaD or AD by other putative risk factors for cognitive decline, we finally repeated all analyses adding an interaction term to the model that contained variables for incident stroke (yes/no) and the individual risk factor. Risk factors for cognitive decline assessed in these analyses were diabetes mellitus, APOEε4 genotype, systolic and diastolic blood pressure, serum CRP levels, body mass index, and IMT. The time-to-event variable in all models was age at onset of dementia, VaD and AD, death or end of follow-up, respectively. Individuals who developed dementia, VaD or AD before incident stroke were censored at time of dementia diagnosis. Persons who did not develop dementia, who died, or who were lost to follow-up owing to relocation before development of dementia were censored at the time of their last evaluation. Because the distribution of serum CRP levels was skewed, logarithmic transformation of this variable was carried out before analyses were performed. All data analysis was performed using SPSS version 13.0 software (SPSS Inc, Chicago, Ill).

RESULTS

There were 6724 persons without dementia or stroke at baseline, with 49,361 person-years of follow-up (mean 7.3 person-years, SD 4.3 person-years). From these 6724 individuals, 713 persons (10.6%) had a stroke during follow-up, and 55 persons subsequently developed dementia after stroke (8.3% of persons with incident stroke). Out of those 55 persons with dementia, 32 (58.2%) were diagnosed with VaD, and18 (32.7%) were diagnosed with AD. During follow-up 627 persons (9.7%) developed dementia without previously having a stroke.

The baseline demographic and clinical characteristics of the study sample are shown in table 1. In Kaplan-Meier-Analyses, the cumulative proportion of survivors without dementia at the end of the follow-up period was 91.3%. The cumulative proportion of survivors without dementia at the end of the follow-up period was 87.8% in the group with a MMSE score of < 25 at last follow-up before incident stroke, and 92.5% in the group with a MMSE score of >= 25 at last follow-up before stroke (p=0.6). The cumulative proportion in the group without incident stroke was 97.6% (Figure).

In cox proportional hazards analyses, persons with incident stroke had a significantly higher risk of subsequent dementia than persons remaining free of stroke during follow-up (age and sex adjusted HR 2.1, 95% CI 1.55-2.81, p<0.0001; table 2). This association remained stable in

Table 1. Baseline characteristics of the study sample in 6724 persons followed prospectively

Women, n (%)	4033 (60.0)
Age, mean (SD), year	69.2 (8.9)
Educational level	
Low	2493 (37.1)
Intermediate	1797 (26.7)
High	2320 (34.5)
APOEε 4/- or 4/4 genotype, n (%)	1724 (25.6)
MMSE score, mean (SD)	27.7 (1.9)
Diabetes mellitus, n (%)	672 (10.0)
Systolic blood pressure (mmHg), (SD)	139.1 (22.3)
Diastolic blood pressure (mmHg), mean (SD)	73.8 (11.4)
Body mass index (kg/m^2), mean (SD)	26.3 (3.9)
CRP (mg/l), mean (SD)	3.3 (6.7)
Total cholesterol (mg/dl), mean (SD)	256.4 (47.0)
HDL (mg/dl), mean (SD)	52.1 (13.9)
Smoking, n (%)	4281 (63.7)
Intima media thickness (mm), mean (SD)	0.8 (0.2)

MMSE: Mini Mental State Examination. CRP = C-reactive protein. HDL = high-density lipoprotein (HDL) cholesterol

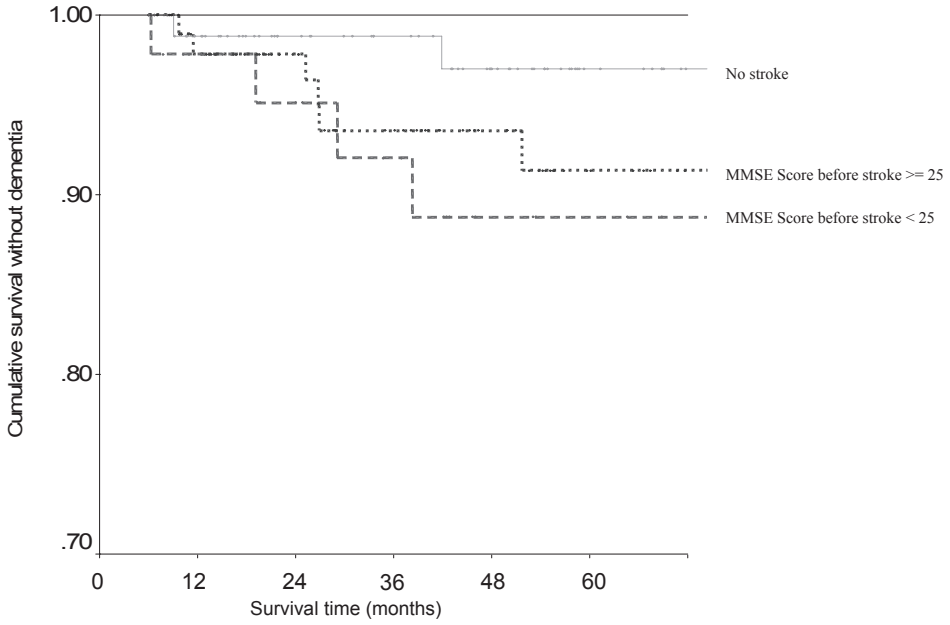

Figure. Age and sex adjusted cumulative proportion of persons surviving without developing dementia, as a function of the last MMSE score before incident stroke

models in which we additionally adjusted for APOE genotype and education. The magnitude of this association also did not change in models to which the pre-stroke measures of cognitive function or other potential risk factors for cognitive decline were added (table 2).

To test for an interactive effect between incident stroke and pre-stroke level of cognitive function on the risk of subsequent dementia, we then repeated all analyses adding an interaction term to the model that contained variables for incident stroke (yes/no) and pre-stroke cognitive performance. There was no interactive effect of incident stroke and measures of pre-stroke cognitive function on the risk of subsequent dementia in these analyses (HR 1.3, 95% CI 0.75-1.62, p=0.7).

When we repeated the analyses restricted to VaD only as the outcome, the risk of VaD after incident stroke was four-fold increased (HR 4.0, 95% CI 2.82-6.19). The magnitude of this association also did not change in models to which the pre-stroke measures of cognitive function or other potential risk factors for cognitive decline were added (table 2), and there was no significant interactive effect of incident stroke and pre-stroke cognitive performance on the risk of VaD (HR 1.1, 9% CI 0.65-1.69), p=0.8).

We then repeated all models restricted to AD only as the outcome. There was no association between incident stroke and the risk of subsequent AD (HR 1.3, 95% CI 0.87-1.98, p=0.4,

Table 2. Hazard ratios and 95% confidence intervals, relating incident stroke, and clusters of incident stroke with baseline measures of risk factors for cognitive decline, with the risk of incident dementia, VaD and AD

Variable	Dementia		VaD		AD	
	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Incident stroke	2.1 (1.55-2.81)	2.1 (1.54-2.93)	4.0 (2.82-6.19)	4.0 (2.82-6.19)	1.3 (0.87-1.98)	1.4 (0.89-2.14)
+ MMSE	2.0 (1.45-2.69)	1.9 (1.36-2.68)	3.9 (2.75-6.17)	3.8 (2.61-6.09)	1.2 (0.71-1.77)	1.1 (0.67-1.79)
+ rate of decline in MMSE over time ††	2.0 (1.41-2.87)	2.0 (1.34-2.87)	3.9 (2.76-6.18)	3.8 (2.62-6.10)	1.2 (0.72-1.78)	1.1 (0.66-1.79)
+ APOEε4 genotype	2.1 (1.53-2.84)	2.1 (1.54-2.93)	4.0 (2.81-6.18)	4.0 (2.79-6.19)	1.3 (0.87-1.98)	1.4 (0.89-2.14)
+ diabetes	2.1 (1.55-2.81)	2.1 (1.54-2.93)	4.0 (2.82-6.19)	4.0 (2.83-6.20)	1.3 (0.89-2.04)	1.4 (0.89-2.14)
+ systolic blood pressure	2.1 (1.52-2.82)	2.0 (1.43-2.78)	4.0 (2.79-6.32)	3.9 (2.65-6.22)	1.3 (0.87-2.04)	1.3 (0.82-2.04)
+ diastolic blood pressure	2.1 (1.51-2.81)	2.0 (1.44-2.81)	4.0 (2.68-6.35)	3.9 (2.64-6.26)	1.4 (0.88-2.05)	1.3 (0.84-2.06)
+ serum CRP †	2.0 (1.47-2.81)	1.9 (1.37-2.77)	3.9 (2.74-6.15)	3.8 (2.64-6.05)	1.4 (0.91-2.16)	1.4 (0.86-2.16)
+ total cholesterol	2.2 (1.59-2.91)	2.2 (1.57-2.99)	4.1 (2.85-6.25)	4.1 (2.81-6.20)	1.4 (0.92-2.10)	1.4 (0.92-2.18)
+ HDL	2.2 (1.61-2.92)	2.1 (1.54-2.93)	4.1 (2.84-6.24)	4.0 (2.69-6.13)	1.4 (0.93-2.12)	1.4 (0.87-2.14)
+ smoking	2.1 (1.54-2.81)	2.1 (1.55-2.95)	4.0 (2.82-6.19)	4.0 (2.75-6.15)	1.3 (0.83-1.94)	1.4 (0.88-2.13)
+ IMT	2.2 (1.54-3.99)	2.1 (1.46-4.19)	4.1 (2.86-6.24)	4.0 (2.68-6.13)	1.3 (0.74-2.06)	1.2 (0.71-2.04)

Cox proportional hazards model. HR=hazard ratio, 95% CI= 95 percent confidence interval. Model 1: adjusted for gender and age; Model 2: adjusted for gender, age, education and APOEε4 genotype. MMSE = Mini Mental State Examination; CRP = C-reactive protein; HDL = high-density lipoprotein (HDL) cholesterol; IMT = Intima media thickness; † serum CRP was used as a logarithmic transformed continuous variable; †† beta coefficient for rate of decline in MMSE score over time before occurrence of incident stroke, derived by linear regression

table 2). There was also no significant interactive effect of incident stroke and pre-stroke cognitive performance on the risk of AD (HR 1.1, 95 CI 0.72-1.58, p=0.6).

To test for an interactive effect between incident stroke and baseline measures of other common risk factors for cognitive decline on the risk of subsequent dementia, VaD or AD, we finally repeated all analyses adding an interaction term to the model that contained variables for incident stroke (yes/no) and the respective risk factor. In these analyses, none of the assessed interaction terms was associated with the risk of subsequent dementia, VaD or AD.

DISCUSSION

In this study we found that an incident stroke doubles the risk of subsequent dementia, and four-folds the risk of subsequent VaD, independent of pre-stroke level of cognitive function and pre-stroke rate of cognitive decline. When the analyses were restricted to AD only as the outcome, the association was attenuated.

This study has limitations. First, we restricted our analyses to persons with complete information on the occurrence of incident stroke. Individuals which were excluded due to incomplete information were older and had a higher frequency of different vascular risk factors. However, it seems unlikely that in these persons the association between incident stroke, pre-stroke cognitive function and dementia was opposite to what we found. Second, some of the stroke diagnoses were made without imaging of the brain, leaving a possibility of misclassification of stroke. However, if misclassification has occurred, it is likely to be non-differential, leading to an underestimation of the observed effects.

An important strength of our study is that it is a prospective population-based study with a large total number of participants, a large number of persons with incident stroke during follow-up, and nearly complete follow-up with respect to incident stroke and subsequent dementia. Previous studies relating stroke with the risk of dementia were mostly observational studies using prevalent information of stroke, or studies assessing cognitive deterioration after acute stroke in clinical settings.[9,10,12-14,27,38] To our knowledge, this is the first large population-based study relating incident stroke with the long-term risk of subsequent dementia in persons without dementia or stroke at baseline. This design provides the ability to explore the impact of stroke and other risk factors on the risk of dementia explicitly taking pre-stroke cognitive performance into account.

We observed an association between incident stroke and the risk of subsequent dementia, which was independent of level of pre-stroke cognitive performance. This finding contradicts previous studies reporting a higher risk of PSD in persons with pre-stroke cognitive impairment compared with persons with normal cognition before stroke.[11,13,14,27] However, as mentioned before, these studies either used prevalent information of stroke,[11,13,16,38] or were conducted in stroke cohorts with pre-stroke cognitive function being measured after the stroke through informant questionnaires or by checking pre-stroke medical records for a diagnosis of dementia.[4,9,12,14,27] These studies thus lacked the ability to accurately assess pre-stroke cognitive function.[4,5,8-10,12,27,38] Also, due to the difficulties in applying a comprehensive, formal neuropsychological assessment to patients who are physically and neurologically impaired, many of the studies in clinical settings examined only a subsample of the total patients registered,[4,5,9,10] and thus may have been biased due to selective attrition.[39] The present study with a mean follow-up time of 6.3 years between first assessment of cognitive function at baseline and time of incident first stroke, in which also the slope of cognitive performance before stroke could be taken into account, and which had a nearly complete follow-up with respect to dementia, does not suggest that the pre-stroke level of cognitive function is a major determinant of the effect of stroke on the risk of PSD.

The association between incident stroke and the risk of subsequent dementia was also independent from all other assessed risk factors for cognitive decline, including diabetes mellitus, APOEε4 genotype, blood pressure levels, body mass index, and IMT. This observation supports the notion that the effects of stroke result in dementia through mechanisms other than mechanisms of APOEε4 or other potential risk factors, and that stroke increases the risk of dementia independently of these risk factors.

When the analyses were restricted to AD only as the outcome, the association between incident stroke and the risk of subsequent AD was strongly attenuated and became non-significant. This finding seems reasonable since persons with a diagnosis of stroke are, by definition, more likely to receive a diagnosis of VaD rather than AD.

There are alternative explanations for our findings. It is possible that incident stroke is not a risk factor but merely part of a pre-clinical syndrome of dementia, meaning that persons with pre-clinical dementia may have a higher frequency of stroke than persons without dementia. However, the mean follow-up time between incident stroke and subsequent dementia in persons developing PSD was relatively long (3.9 years), making this an unlikely explanation for our findings. Also, the association between incident stroke and subsequent dementia was independent from pre-stroke cognitive function, regardless of length of follow-up time from incident stroke to subsequent dementia.

An alternative explanation for the missing interaction between incident stroke and risk factors for cognitive decline might be that elderly cohorts are too homogeneous to show differences in outcomes related to these risk factors. The measurement of these risk factors in our cohort did not take into account duration. Thus, it is possible that our results tend to underestimate the association between incident stroke, risk factors for cognitive decline and incident dementia, which could bias the results to the finding of no interaction. However, this seems unlikely given the strong robustness of our findings across all assessed risk factors for cognitive decline.

In summary, stroke seems to exert its effect on dementia risk independent of the pre-stroke level of cognitive function. It also seems to act through mechanisms other than mechanisms of common potential risk factors for cognitive decline. The association of stroke seems to be stronger with the vascular component of dementia syndromes than with the Alzheimer component.

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5

General Discussion

BACKGROUND AND KEY OBJECTIVE

Background. Over the past years evidence has been accumulating that late-onset dementia is a heterogeneous and multifactorial disorder, and that besides accumulation of beta amyloid and neurofibrillary tangles, other factors, in particular vascular risk factors and cerebrovascular disease, may be involved in its etiology. In autopsy studies, 15-35% of the brains of elderly persons, who had been diagnosed with dementia during their lifetime, had not only a higher burden of amyloid plaques and neurofibrillar tangles but rather a mixed pathology also consisting of significant cerebrovascular disease.[1] Several observational and clinical studies reported associations between various vascular risk factors or measures of cerebrovascular disease and cognitive decline or dementia.[1-8]

Dementia has a long pre-clinical period. Further, it is uncertain whether factors that are more frequently observed in persons with dementia than non-demented persons, such as vascular risk factors or cerebrovascular disease, are causally involved in dementia etiology or simply reflect coexisting disease common in the elderly. These facts demand studies with a long follow-up time to achieve the ability to disentangle causes and consequences in the association between vascular disease and cognitive decline.

Most of the previous studies relating vascular risk factors with the risk of cognitive decline and dementia, however, had cross-sectional study designs or short follow-up periods implying the potential to include persons with pre-clinical disease at baseline or to lack enough incident dementia cases to achieve reliable statistical precision.

The studies relating stroke with the risk of subsequent cognitive decline or dementia, were either performed in observational cohorts using prevalent information on stroke,[5,9,10] or were conducted in stroke cohorts with pre-stroke cognitive function being measured after the stroke through informant questionnaires or by checking pre-stroke medical records for a diagnosis of dementia.[3,4,11] These studies thus lacked the ability to take accurately assessed pre-stroke cognitive function into account. Also, due to the difficulties in applying a comprehensive, formal neuropsychological assessment to patients who are physically and neurologically impaired, many of the studies in clinical settings examined only a subsample of the total patients registered, and thus may have been biased due to selective attrition.[12]

Key objective. The objective of the work described in this thesis was to gain more insight into vascular and genetic risk factors underlying dementia etiology, and to further clarify the impact of cerebrovascular disease on the risk of cognitive decline taking pre-stroke cognitive function into account. I sought to do this by exploring the association between vascular risk factors and cerebrovascular disease with cognitive impairment in three independent cohorts with long follow-up or quantitative brain imaging.

In this chapter I will summarize the work described in this thesis and discuss its implications. I will first review the pathophysiological main hypotheses linking vascular disease with cognitive impairment and dementia. Then I will review the characteristics of the study populations used, and will discuss the main findings in the light of current knowledge on etiology of dementia and under consideration of potential methodological limitations. Finally, I will comment on the clinical implications of the findings and will suggest directions for future research.

MAIN HYPOTHESES LINKING VASCULAR DISEASE AND DEMENTIA

Based on the literature, there are at least five mechanisms that may underlie the association between vascular risk factors or cerebrovascular disease and dementia (figure). These are not necessarily mutually exclusive.

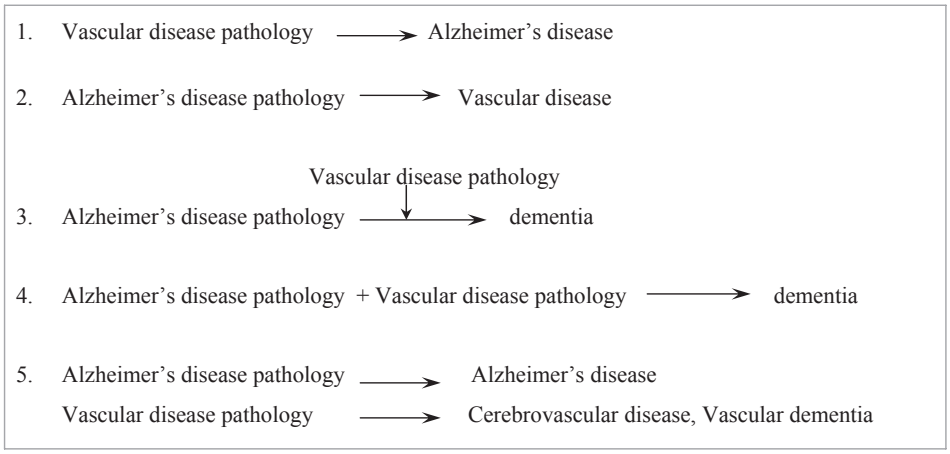

Figure. Main hypotheses linking vascular disease, dementia and Alzheimer's disease

1. Vascular disease causes Alzheimer's disease. Vascular risk factors can lead through cerebrovascular disease to reduced cerebral perfusion. This in turn may affect microcirculation and delivery of energy substrates required for optimal brain cell function. Disturbances in the glucose-oxygen delivery to neurons can lead to abnormal protein synthesis and eventually to production of senile plaques and neurofibrillary tangles.[13,14] Individual vascular risk factors may also have individual pathways leading to dementia. Brain cholesterol may alter the degradation of amyloid precursor protein,[15] which contributes to the pathogenesis of Alzheimer's disease. Smoking may augment cholinergic metabolism by upregulation of cholinergic nicotinic receptors in the brain.[16] Cholinergic deficits, characterized by reduced levels of acetylcholine and nicotinic receptors, are found in Alzheimer's disease.[17] Hypertension may

contribute to a blood-brain barrier dysfunction, which has been suggested to be involved in the etiology of Alzheimer's disease.[18] Besides increasing the risk of cognitive decline by increasing the deposition of amyloid β, manifest stroke may cause cognitive decline by destruction of brain parenchyma and atrophy,[19,20] or by causing damage in strategic locations that lead to amnestic syndromes.[14]

2. Alzheimer's disease causes vascular disease. There is also evidence that the relation may be inverse, and that Alzheimer's disease pathology may contribute to the pathogenesis of vascular disease. Deposited β-amyloid has a toxic effect on the vascular endothelium.[21] This may lead to an up-regulation of cytokines and adhesion molecules, leukocytes, and platelet margination and transmigration, which in turn can lead to vascular damage and hemostatic alterations that predispose to thrombosis, inflammation and ischemia.[22]

3. Interaction between Alzheimer's disease pathology and vascular disease. Alzheimer's disease pathology and vascular disease may trigger each other in bringing out the dementia syndrome. Vascular disorders, such as chronic hypertension or smoking, are associated with an increased vascular permeability with protein extravasation.[23] This may lead to an interference of the extravasated proteins with neuronal function, which then may lead to amyloid accumulation. Amyloid was found to be distributed in the proximity to blood vessels, supporting the hypothesis that its origin may be vascular.[24]

4. Additive effect of Alzheimer's disease pathology and vascular disease. It is also possible that Alzheimer's disease pathology and vascular disease do not interact or cause each other, but rather precipitate and cause cognitive impairment in an additive manner when a certain threshold is reached.

5. Co-existence of Alzheimer's disease and cerebrovascular disease (co-morbidity): Finally, it is possible that Alzheimer's disease pathology and cerebrovascular disease within one person are simply unrelated common co-existing pathologies in the elderly.

In the light of current knowledge and the findings derived from the work described in this thesis, these mechanisms are not equally likely. The strong evidence for a rather mixed pathology with amyloid plaques, neurofibrillary tangles and significant cerebrovascular disease in persons with dementia as well as findings from recent imaging studies reporting associations between several measures of clinical and subclinical cerebrovascular disease and a higher risk of dementia, suggest that vascular disease in fact increases the risk of dementia. This is further supported by recent observational studies reporting associations between vascular disease in mid-life and a higher risk of dementia in late-life. In the work described in this thesis I assessed the impact of several vascular risk factors and cerebrovascular disease on the risk

of dementia, and explored the interactive effect of stroke and pre-stroke cognitive function on dementia risk. My work strongly supports the hypothesis that vascular disease increases the risk of dementia, and it further suggests that mechanisms involved in tissue response to cerebrovascular disease may play a key role in the association between cerebrovascular disease and cognitive decline. I will give a detailed discussion of the study populations and the main findings of my work in the following.

STUDY POPULATIONS USED IN THIS WORK

I explored the association of vascular disease with cognitive decline in three independent cohorts: the Rotterdam Study by the Department of Epidemiology & Biostatistics at Erasmus Medical Center Rotterdam; the Rotterdam Scan Study by the Department of Epidemiology & Biostatistics at Erasmus Medical Center Rotterdam; and the Washington Heights Inwood Columbia Aging Project (WHICAP) by the G.H. Sergievsky Center at Columbia University, New York.

The *Rotterdam Study* is a population-based prospective study among 7,983 residents of Onmoord, a district of the city of Rotterdam, aged 55 years or older, that investigates the incidence and causes of cardiovascular, neurodegenerative, locomotor, and ophthalmologic diseases in the elderly.[25] All participants underwent at baseline in 1990 and at all follow-up examinations (1993-1994, 1997-1999, 2002-2004) a protocol for assessment of cognitive function. In addition, the cohort was continuously monitored for incident dementia and stroke cases. During this time, 743 incident dementia cases were identified. Follow-up with respect to dementia was virtually complete (99.9%).

The *Rotterdam Scan Study* is a population-based prospective MRI study among 1,077 non-demented persons aged 60 to 90 years that was designed to explore causes and consequences of brain changes on MRI in the elderly.[26] All participants underwent a brain MRI in 1995 to 1996, 668 participants (62%) underwent a second MRI at three-year follow-up. The severity of white matter lesions and the presence of brain infarcts were measured. In addition, all participants underwent a structured interview, physical examination, and neuropsychological testing at baseline and all follow-up examinations.

The *WHICAP Study* is a prospective cohort study among 4,316 randomly sampled Medicare recipients 65 years or older and residing in northern Manhattan, that was designed to identify cognitive decline and its causes in elderly persons.[27] The participants were recruited at two time periods, 2,126 participants were recruited in 1992-1994 and 2,190 participants were recruited in 1999-2002. They have been followed up at approximately 18-month intervals

with similar assessments of general health and function, medical history, physical and neurological examination, and a detailed neuropsychological assessment at each follow-up. For the longitudinal studies described in this work, only participants from the 1992 cohort were included in the analyses. During follow-up of these cohorts, 270 incident dementia cases were identified.

REVIEW AND INTERPRETATION OF MAIN FINDINGS

The objective of the work described in this thesis was to gain more insight into vascular and genetic risk factors underlying dementia etiology, and to further elucidate the impact of cerebrovascular disease on the risk of cognitive decline. I addressed this research question using three approaches. First, I related various vascular risk factors (plasma lipid levels, hypertension, and smoking habit) with different stages of cognitive decline.

Then, I assessed the impact of variation in genes encoding C-reactive protein and Matrix metalloproteinase 3, which are involved in inflammation and vascular pathology, on the risk of cognitive impairment and cerebral small-vessel disease. Cerebral small-vessel disease is associated with an increased risk of cognitive decline and dementia,[6,7,28] and may be an intermediate step between vascular disease and cognitive impairment. Study of the association of variation in genes encoding for vascular risk factors with vascular disease and cognitive decline provides the ability to elucidate the role of these risk factors taking residual confounding into account.

Finally, I explored the direct impact of cerebrovascular disease on the risk of cognitive impairment and dementia in persons free of dementia at baseline taking pre-stroke cognitive impairment into account.

1. Vascular risk factors and cognitive decline

Plasma lipid levels. First, I explored the association of late-life plasma lipid levels with different stages of cognitive impairment in the WHICAP Study. In longitudinal analyses, there was a weak association between elevated levels of low-density lipoprotein cholesterol and non-high density lipoprotein cholesterol with the risk of vascular dementia, and a weak association between elevated levels of total cholesterol and a decreased risk of Alzheimer's disease. There was no association between levels of any other plasma lipid and Alzheimer's disease, plasma lipid levels and memory performance over time, or plasma lipid levels and amnestic or non-amnestic forms of mild cognitive impairment (MCI). There was no association between intake of lipid lowering medication and cognitive status.

In summary, these observations do not support the hypothesis that plasma lipid levels in late-life are implicated in the Alzheimer's disease component of dementia. This is in line with findings of no association by other studies and the WHICAP study after shorter follow-up.[29,30]

However, it is important to bear in mind that this does not contradict studies reporting a relation between early- or mid-life plasma lipid levels and risk of dementia, [31,32] since effects of plasma lipid levels on vascular disease, brain metabolism or amyloid deposition may change during life, and older persons have a higher prevalence of certain conditions that can affect plasma lipid levels, including occult illness, inflammation, or weight loss.[33]

Smoking. In analyses relating smoking status with the risk of dementia and Alzheimer's disease in the Rotterdam Study, there was an association between current smoking and an increased risk of dementia and Alzheimer's disease in persons without the APOEε4 allele. This is in line with the finding of a faster decline over time explicitly in memory but not executive or language performance in current smokers without the APOEε4 allele in the WHICAP study, and is also consistent with previous findings in the Rotterdam Study of an increased risk of dementia and Alzheimer's disease in persons without the APOEε4 allele after a mean follow-up time of 2.1 years.[34] It is possible that individuals who carry the APOE-ε4 allele have an increased risk of Alzheimer's disease in such a way that other risk factors do not increase the risk further, or that smoking is harmful through vascular mechanisms, but also partly beneficial in APOEε4 carriers. The latter hypothesis is supported by previous findings that persons with Alzheimer's disease who are APOEε4 carriers have fewer nicotinic receptor binding sites and lower activity of choline acetyltransferase than non-carriers.[35] Smoking could counterbalance the APOEε4 associated impairment by facilitating the release of acetylcholine or increasing the density of nicotine receptors. However, in our study there was no interactive effect of (current) smoking and APOEε4 genotype on the risk of dementia or Alzheimer's disease. Also, APOEε4 carriers who smoked had - if any- a higher risk of dementia than APOEε4 carriers who never smoked. These facts rather support the hypothesis that smoking in fact increases the risk of dementia, but that this effect is less pronounced in persons who already are at increased risk by having an APOEε4 genotype.

Hypertension. In the analyses relating hypertension with the risk of mild cognitive impairment (MCI) in the WHICAP study, hypertension was related to a higher risk of all-cause and non-amnestic MCI, but not amnestic MCI. MCI has been described as an intermediate stage between normal cognition and dementia.[36,37] Non-amnestic MCI, as defined in the present study, may be related in particular to cerebrovascular disease and vascular dementia. Since hypertension is associated with a higher risk of cerebrovascular disease and vascular dementia, it seems reasonable that it must be related with the risk of non-amnestic MCI. Also, the relation of hypertension to non-amnestic MCI remained stable after adjusting for education, race and APOEε4 genotype and was attenuated after adjustment for stroke and vascular risk factors, further suggesting that cerebrovascular disease may be mediating the relation between hypertension and non-Alzheimer's disease forms of MCI. In the interpretation of these findings it is of major importance to keep in mind that MCI is likely to be a clinically

and pathologically heterogeneous syndrome, and that definitions of MCI and MCI subtypes are not clearly established diagnostic entities. The frequency of dementia in a group of individuals with cognitive impairment is the result of both the definition of the disorder and the underlying pathophysiology. Different definition of MCI or MCI subtypes may lead to different results. Our study thus does not exclude the possibility that hypertension is associated with a type of MCI that is related to the Alzheimer component of dementia.

It is important to note that during the course of this work, blood pressure has been studied in several studies and in multiple measures of brain aging, and that the results have been inconsistent. Closer analysis of the inconsistencies between the studies suggests that the discrepancies among them may be accounted for by elements of the study design, specifically a combination of the duration of time between the measurement of blood pressure and brain function, and the ages at which the measurements were made. In general, it seems that the older the age the blood pressure is measured, and the shorter the interval between the measure of blood pressure and brain function, the more difficult it is to investigate whether the risk for cognitive impairment is altered by levels of blood pressure. The reason for this may be that blood pressure declines with age - or more specifically - with age-related pathology, such as vessel stiffening, weight loss, and changes in the autonomic regulation of blood flow. However, it is also possible that the effects of blood pressure on cognitive function simply differ between older and younger persons.

2. Genetic polymorphisms and risk of dementia and cerebral small-vessel disease

In the second approach, I assessed the impact of variation in genes encoding C-reactive protein (CRP) and Matrix metalloproteinase 3 (MMP-3) on the risk of cognitive impairment and cerebral small-vessel disease.

Variation in the CRP-gene and risk of cerebral small-vessel disease. Plasma levels of CRP had previously been observed to be associated with measures of cerebral-small vessel disease and cardiovascular disease in the Rotterdam Study as well as other studies.[38-40] Since plasma levels of CRP are influenced by several factors (such as obesity, smoking, adverse socioeconomic circumstances and various disease states) that are associated with the risk of vascular disease itself, it remained unclear whether these associations were true associations or caused by residual confounding. To explore whether CRP is causally involved in cerebral small-vessel disease, which in turn may be an intermediate stage to cognitive decline, I related haplotypes, representing the common genetic variation in the gene encoding CRP, with measures of cerebral small-vessel disease. Common variation in the CRP–gene was neither in the Rotterdam Scan Study nor in the independent population of the 'Memory and Morbidity in Augsburg Elderly' (MEMO) Study associated with white matter hyperintensities or lacunar infarcts. This

finding does not support the hypothesis that CRP plays a causal role in the pathogenesis of small-vessel disease.

Variation in the MMP-3 gene and risk of dementia and Alzheimer's disease. Findings of recent animal and post mortem studies suggested that MMPs, a family of zinc- and calcium-dependent endopeptidases that are involved in the degradation of connective tissue and extracellular matrix, may be implicated in the pathogenesis of Alzheimer's disease.[41-44] MMP-3 is directly involved in Aß degradation,[41,42] and there is evidence that it has a reduced expression in Alzheimer's disease hippocampi, suggesting that it plays a role in selective neurodegeneration. MMP-3 is furthermore involved in the pathogenesis of atherosclerosis; common polymorphisms in the gene encoding MMP-3, in particular the 5A6A promotor polymorphism, have been repeatedly associated with the risk of atherosclerosis and cardiovascular disease,[43,44] which in turn may be associated with the risk of cognitive impairment. I sought to explore whether these associations can be replicated in a population-based sample, and explored in the Rotterdam Study and the Rotterdam Scan Study the association of MMP-3 haplotypes with dementia and hippocampus volume. There was no association between variation in the MMP-3 gene and dementia or hippocampus volume. These findings do not suggest that MMP-3 plays a causal role in the pathogenesis of dementia.

3. Stroke and cognitive decline

Finally, I explored the direct impact of cerebrovascular disease on the risk of cognitive impairment and dementia in persons free of dementia at baseline. First I related stroke with cognitive performance over time in WHICAP. In these analyses, stroke was related to a progressive decline in memory and abstract/visuospatial performance especially among men and those without an APOEε4 allele. In analyses relating incident stroke as a time-varying exposure with the risk of dementia in the Rotterdam Study, incident stroke was associated with a more than doubled risk of subsequent dementia, independent of pre-stroke cognitive performance and other potential risk factors for cognitive decline. This finding contradicts previous studies reporting a higher risk of post-stroke dementia in persons with pre-stroke cognitive impairment compared with persons with normal cognition before stroke.[1,12,45,46] However, these studies either used prevalent information on stroke or were conducted in stroke cohorts with pre-stroke cognitive function being measured after the stroke through informant questionnaires or by checking pre-stroke medical records for a diagnosis of dementia, and thus lacked the ability to take accurately assessed pre-stroke cognitive function into account. Our study had a mean follow-up time of 6.3 years between first assessment of cognitive function at baseline and time of incident first stroke and took also the slope of cognitive performance before stroke into account. Our findings do not suggest that the pre-stroke level of cognitive function is a major determinant of the effect of stroke on the risk of post-stroke dementia.

METHODOLOGICAL CONSIDERATIONS

In this work I aimed to explore the impact of genetic and vascular risk factors and cerebrovascular disease on the risk of cognitive decline and dementia. The ability of identifying factors involved in dementia etiology depends on the precision of measurements, the validity of the study results, and the possibility of causal inference from these results. In the previous chapters I have discussed the methodological considerations of each study separately. In this section, I will give an overall review of the methodological strengths and limitations specifically concerning the studies described in this thesis, and will then briefly address a methodological issue that in general has to be considered in dementia research: the distinction between subtypes of dementia. For an overview of general methodological considerations in epidemiological research I refer to a standard text.[47]

Strengths of the studies in this thesis. The studies described in this thesis were performed in three independent large population-based cohort studies. Studying causes of a disease in independent datasets can provide arguments for the causality of the studied association. A strength of a longitudinal population-based design is that, provided that the response is high enough and loss to follow-up is minimized, selection bias is limited. Another advantage of our study design is that the results can be generalized to the general population. The prospective nature of the studies described in chapter 2 and 4 allowed to establish a temporal relationship between vascular disease and dementia, which can provide arguments for causality. The study of genetic variation in candidate genes, as in chapter 3, can provide arguments for causality by making use of Mendelian Randomization.

Limitations of the studies in this thesis. In general, a large sample size is the primary way to increase precision in an epidemiological study. However, precision also relates to the efficiency of a study, and despite the large sizes of all study cohorts, the number of people who developed incident dementia or had the genetic variation of interest was modest. Therefore, precision was a concern in our study, especially in the analyses relating genetic variation in the genes encoding CRP and MMP-3 with dementia and cerebral small-vessel disease (Chapter3).

A frequent methodological issue in observational studies on cognitive impairment and dementia is subject attrition. Persons with cognitive impairment as well as persons who are generally less healthy, are more likely to drop out of the study and thus not participate in a follow-up examination.[48] In both the Rotterdam Study and the WHICAP Study persons with cognitive impairment or a higher burden of vascular disease were slightly more likely to be lost to follow-up than persons without cognitive impairment or a lower burden of vascular disease. This selection may have resulted in an underestimation of the associations between vascular risk factors and cerebrovascular disease and dementia.

Genetic association studies have further methodological characteristics that have to be considered. Because linkage disequilibrium (LD) is sustained over only a short chromosomal segment, a large number of loci need to be tested to cover a region, which in turn increases the possibility of false-positive findings. One cannot rely on the conventional threshold p-value of 0.05. With each test, the possibility of a false-positive result increases, requiring the need either for replication in an independent study or computer simulation. Another important factor is the difference between the disease allele frequency and the frequency of either the single SNP or haplotype that is in linkage disequilibrium with the disease allele. It has been shown that power is a function of both linkage disequilibrium between disease allele and the marker allele or haplotype, and the difference of the disease allele frequency and the frequency of the marker allele or haplotype in linkage disequilibrium with the disease allele. Power is maximal when linkage disequilibrium is maximal and the frequency difference is 0. Also, if heritability or relative risk of the trait is low, the power to detect a small effect is also low. For case-control studies, the persons with disease and the comparison group of controls can differ in genetic background, introducing variables unrelated to the disease and causing a type of spurious association or confounding (population stratification). In the studies described in this thesis, we tried to address these issues by ensuring an ethnically homogeneous sample, a large enough sample size to increase precision and minimize random error, coverage of the complete genetic variation of the gene of interest by proper genotyping of tagging SNPs, and where applicable, replication of the findings in an independent sample.

Distinction between subtypes of dementia. The distinction between Alzheimer's disease with vascular pathology and vascular dementia is very difficult and may be even impossible. There is little agreement on what should be the criteria for vascular dementia, and the criteria that are being used show bad reproducibility.[49] Although the NINCDS-ADRDA criteria for Alzheimer's disease are widely accepted and show good reproducibility,[49] they do not guarantee that the clinical syndrome of Alzheimer's disease refers to one clearly delineated disease entity. Dementia may actually be one entity with a mixture of Alzheimer's pathology and cerebrovascular pathology. Until there are better tools to distinguish between subtypes of dementia, if existing, it may be more informative to consider overall dementia and to distinguish according to etiology, without the assumption that these subgroups indeed reflect clearly separable diagnostic entities.

CLINICAL IMPLICATIONS OF THE FINDINGS FROM THIS WORK

Research on vascular disease in the etiology of dementia is driven by the idea that it might offer clues for treatment or preventive intervention. Although observational epidemiological studies are not specifically designed to give guidelines for clinical practice, I will comment

in this section on the clinical implications the findings of this work may have. I will do this separately for each risk factor assessed. Since – as described above - vascular dementia and Alzheimer's disease are up to date not clearly delineated disease entities and may in fact have a common underlying cause with a mixture of vascular disease and Alzheimer's disease pathology, I will discuss the implications of the findings for *overall* dementia rather than for vascular dementia and Alzheimer's disease separately.

Plasma lipids. The studies relating plasma lipid levels with cognitive decline over time, mild cognitive impairment and dementia (Chapter 2), showed a weak association between elevated levels of low-density lipoprotein cholesterol and non-high density lipoprotein cholesterol with the risk of vascular dementia, and a weak association between elevated levels of total cholesterol and a decreased risk of Alzheimer's disease. There was no association between levels of any other plasma lipid and Alzheimer's disease, plasma lipid levels and performance in any cognitive domain over time, or plasma lipid levels and amnestic or non-amnestic forms of mild cognitive impairment (MCI). There was no association between intake of lipid lowering treatment and risk of dementia. From these findings one might conclude that lipid lowering treatment would not have an overall effect on the incidence of dementia. However, one has to bear in mind that I looked at the effect of late-life plasma lipids and late-life plasma lipid lowering treatment. Studies exploring the effects of plasma lipids in early and mid-life rather suggest a harmful effect of plasma lipids on the risk of cognitive impairment and dementia.[31,32] The association between plasma lipids and dementia needs further clarification before clear clinical implications can be drawn.

Smoking. Current smoking was in two different cohorts associated with an increased risk of dementia and Alzheimer's disease and a faster decline over time explicitly in memory performance in persons without the APOEε4 allele. This finding can have two potential explanations. Either individuals who carry the APOE-ε4 allele have an increased risk of Alzheimer's disease in such a way that smoking does not increase the risk further, or smoking is harmful through vascular mechanisms, but partly beneficial in APOEε4 carriers, for example through counterbalancing the APOEε4 associated impairment by facilitating the release of acetylcholine or increasing the density of nicotine receptors. Even if smoking is partly beneficial in APOEε4 carriers and the mechanism by which nicotine (or conceivably some other chemical substance in cigarettes) mediates this effect can be established, smoking can - due to its adverse effects on several other conditions- obviously not be recommended. Rather, the selective modulation of nicotinic receptors or other potential pathways involved should be targeted.

Hypertension. In the analyses relating hypertension with the risk of MCI, hypertension was related to a higher risk of all-cause and non-amnestic MCI, but not amnestic MCI. Keeping our definition of MCI subtypes in mind, one might conclude from this study that treatment

of hypertension would have a beneficial effect on the vascular component of dementia. This is supported by studies showing a harmful effect of high blood pressure in early and mid-life on the risk of cognitive impairment and dementia.[18,31] However, some recent studies in older cohorts showed a relation between low blood pressure levels and an increased risk of cognitive decline, and low blood pressure and a higher mortality among persons with dementia, respectively. [50-52] Both the age of the patient as well as the stage in the disease process have to be taken into account when antihypertensive intervention for treatment or prevention of dementia is considered.

C-reactive protein. In this work, common variation in the CRP–gene was neither in the Rotterdam Scan Study nor in the independent population of the 'Memory and Morbidity in Augsburg Elderly' (MEMO) Study associated with white matter hyperintensities or lacunar infarcts. This finding does not support the hypothesis that CRP plays a causal role in the pathogenesis of cerebral small-vessel disease. This is supported by several recent studies suggesting that previously reported associations between CRP and vascular disease may have been biased by residual confounding. However, the evidence linking inflammation with vascular disease is strong, and it is possible that CRP is simply the wrong measure in exploration of this association. As long as the association between inflammation and vascular disease is not refuted, anti-inflammatory drugs should be recommended in treatment and prevention of vascular disease.

Matrix metalloproteinase-3. Variation in the MMP-3 gene was not associated with dementia or hippocampus volume. This is in agreement with two other genetic association studies on the relation between MMPs and dementia that have been performed during the course of this work.[53,54] A third, very recent study observed in a case-control sample a borderline significant association between the 6A-allele of the 5A6A promotor polymorphism with an increased risk of dementia in persons without the APOEε4 allele.[55] However, in the same study, this association could not be replicated in a second case-control sample, and there was no association between the 6A allele and subtypes of dementia. In summary, the findings of the four population-based studies do not suggest that intervention in the MMP-3 pathway would have a protective effect on dementia risk in humans. However, the research on the role of matrix metalloproteinases in dementia is in its infancy and more observational and experimental studies are needed before clinical implications can be drawn.

Stroke. Stroke was related to a progressive decline in memory and abstract/visuospatial performance in the WHICAP Study, and a more than doubled risk of subsequent dementia in the Rotterdam Study that was independent of pre-stroke cognitive performance and other potential risk factors for cognitive decline. The latter study was the first to take accurately assessed pre-stroke cognitive function into account. This finding has major implications for

clinical practice since it suggests major benefits of persons with stroke from neuroprotective intervention.

SUGGESTIONS FOR FUTURE RESEARCH

The work in this thesis has provided more insight into the role of several vascular and genetic risk factors in dementia etiology, and has further clarified the impact of cerebrovascular disease on the risk of post-stroke dementia. However, at the same time, it has - together with recent studies by other groups - generated several questions. What is the next step we should take in research on the association between vascular disease and late-onset dementia? How can we further clarify the nature of associations between risk factors and dementia that are difficult to explain by observational studies, such as the seemingly complex associations between blood pressure or lipid levels and cognitive decline? How can we identify the specific factors that determine cognitive function after stroke?

In the work described in chapter 3.1, I related genetic variation in the gene encoding C-reactive protein (CRP) with the risk of cerebral small-vessel disease in the Rotterdam Scan Study, and did not find an association. Prior to this work, a significant association between plasma CRP-levels and cerebral small-vessel disease had been found in the same sample.[38] Several observational studies observed associations between higher plasma CRP levels and various measures of vascular disease or the metabolic syndrome,[40,56-58] but studies relating variation in the CRP gene with these outcomes using a Mendelian randomization approach could not replicate these findings and rather suggested that the previously observed associations had been caused by residual confounding.[59-64] In addition, demonstrating a direct effect of CRP in atherogenesis has been difficult as outlined in a recent report.[65] Findings from several studies relating other endophenotypes with complex outcomes could also not be replicated in studies of variation in genes encoding these phenotypes. Despite the possibility that our study and other genetic association studies may have lacked statistical power to detect a small effect size or did not study rare functional variants possibly associated with the disease, these findings nevertheless raise the question whether in research on complex diseases, such as late-onset dementia, more emphasis should be laid on the study of genetic variation.

Despite several methodological limitations that have to be taken into account (see above), study of variation in genes encoding particularly factors that are influenced by several measures that can not be controlled for in the analyses, could help to reduce spurious findings caused by residual confounding or inverse association. Study of genetic variation would also help to disentangle the nature of associations between certain risk factors and complex outcomes that seem to be difficult to determine by observational studies. The associations

of blood pressure and lipid levels with dementia, for example, remain unclear although in addition to us several groups tried to disentangle these associations in observational studies during the course of this work.[66-72] Both associations seem to be 'age' dependent. While there seems to be a harmful effect of elevated levels of blood pressure and plasma lipids in early-or mid-life on dementia risk, the associations seem to be weaker or even inverse in late-life. Study of genetic variation involved in regulation of blood pressure and lipid levels could help to further clarify these associations. Late-onset dementia might be oligogenic (the cumulative result of variants in several genes), polygenic (the result from a large number of genetic variants, each contributing small effects), or might result from an interaction between genetic variants and environmental risk factors. The merge of modern genome science with population-based, epidemiological research could help to disentangle complex associations between risk factors and late-onset dementia.

A striking finding from this work is the observation that stroke doubles the risk of dementia independent from pre-stroke level of cognitive function. This finding, together with the observation that persons with the same burden of cerebrovascular disease range from no cognitive impairment to severely impaired,[8,73,74] suggests that genes determining tissue response to cerebrovascular disease (eg, genes conveying ischemic tolerance or susceptibility, or the ability to recover from ischemic insult) might be of particular interest in research on late-onset dementia. These genes could be genes affecting an individual's premorbid level of cognitive function (such as genes involved in cholinergic or serotoninergic transmitter systems), genes known to be involved in the Alzheimer's disease pathway (presenilins, amyloid precursor protein, or apolipoprotein E), or specific genes outside these pathways. There is strong evidence from both human and animal studies that variants in the genes for platelet glycoprotein, γ-aminobutyric acid receptors, acid-sensing ion channels, proteases, growth factors and their receptors, transcription factors and α-fibrinogen are involved in neuroprotective or neurodegenrative tissue response to cerebrovascular disease.[75-78] Study of such genes could lead to a better understanding of dementia etiology, and could provide targets for screening, prevention and therapeutic intervention.

Although the candidate gene approach currently seems to be the most robust strategy to identify genetic loci for late-onset dementia, it has a potential disadvantage. It only studies the genetic variation in specific candidate gene(s) in relation to the disease of interest. Genetic loci associated with disease risk but outside and not in linkage disequilibrium with the gene studied remain therefore undetected. Well-performed genome-wide association studies that adequately control for multiple testing and are replicated in independent samples, may be in merge with conventional epidemiological research a good additional approach to detect disease loci not assessed in candidate genes studies.

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6

Summary

SUMMARY (in English)

Dementia is one of the most common neurodegenerative diseases in western societies and a major public health burden. Its prevalence shows an almost exponential increase with age, from about 1% in individuals aged 60-64 years up to 60% in persons 95 years and older.

Amyloid plaques and neurofibrillary tangles are considered the main neuropathological hallmarks of Alzheimer's disease, which is regarded the most frequent subtype of dementia. Recent advances have enabled detailed understanding of the molecular pathogenesis of these changes. However, as the knowledge increases so does evidence that dementia is a heterogeneous and multifactorial disorder, and that besides accumulation of beta amyloid and neurofibrillary tangles, other factors, in particular vascular disease, may be involved, especially in late-onset dementia. Autopsy studies show that 15-35% of the brains of elderly persons, who had been diagnosed with late-onset dementia during their lifetime, have not only a higher burden of amyloid plaques and neurofibrillar tangles but also significant cerebrovascular disease. Observational studies reported associations between several vascular risk factors and cognitive decline and dementia. Clinical stroke has been reported to considerably increase the risk of dementia, with prevalence rates of post-stroke dementia of about 30%.

Most of the evidence relating vascular disease with cognitive decline, however, came from cross-sectional studies, studies with a short follow-up time, autopsy studies, and stroke cohorts not taking accurately assessed pre-stroke cognitive function into account. The long preclinical period of dementia, the pathological diversity that contributes to the clinical symptoms of dementia, and the uncertainty whether factors that are more frequently observed in persons with dementia than non-demented persons, such as vascular risk factors or cerebrovascular disease, in fact cause dementia or simply reflect coexisting disease, demand studies with a longer follow-up period to disentangle causes and consequences in the association between vascular disease and cognitive decline.

The objective of the work described in this thesis was to gain more insight into vascular and genetic risk factors underlying dementia etiology, and to further clarify the impact of cerebrovascular disease on the risk of cognitive decline. I explored data from three independent cohorts that had been designed to explore causes of neurodegenerative and cerebrovascular diseases in the elderly: the Rotterdam Study and the Rotterdam Scan Study by the Department of Epidemiology & Biostatistics at Erasmus Medical Center Rotterdam, and the Washington Heights Inwood Columbia Aging Project (WHICAP) conducted by the G.H. Sergievsky Center at Columbia University, New York.

After introducing the scientific background and the key objective underlying this work in **chapter 1**, I summarize in **chapter 2** studies exploring the association of individual vascular risk factors (plasma lipid levels, smoking, and hypertension) with the risk of different stages of cognitive impairment.

In studies relating *late-life plasma lipid levels* with cognitive function in WHICAP, there was a weak association between elevated levels of low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol and decreased levels of high-density lipoprotein cholesterol with the risk of vascular dementia. Higher levels of total cholesterol were associated with a decreased risk of Alzheimer's disease. Plasma lipid levels were not associated with memory performance over time or mild cognitive impairment (MCI), which is regarded a transitional stage between normal cognition and dementia. In summary, these observations do not support the hypothesis that dyslipidemia in late-life is strongly implicated in the pathogenesis of late-onset dementia.

In analyses relating *smoking habit* with the risk of cognitive impairment, current smoking was in the Rotterdam Study associated with an increased risk of dementia and Alzheimer's disease in persons without the APOEε4 allele, and in WHICAP associated with a faster decline in memory performance over time, especially in men and persons without the APOEε4 allele. It is possible that individuals who carry the APOE-ε4 allele have an increased risk of Alzheimer's disease in such a way that other risk factors do not increase the risk further, or that smoking is harmful through vascular mechanisms, but also partly beneficial in APOEε4 carriers. The latter hypothesis is supported by evidence that persons with Alzheimer's disease who are APOEε4 carriers have fewer nicotinic receptor binding sites and lower activity of choline acetyltransferase than non-carriers. However, in our study there was no interactive effect of smoking and APOEε4 genotype on the risk of dementia or Alzheimer's disease. Also, APOEε4 carriers who smoked had - if any- a higher risk of dementia than APOEε4 carriers who never smoked. These facts rather support the hypothesis that smoking in fact increases the risk of dementia, but that this effect is less pronounced in persons who already are at increased risk by having the APOEε4 genotype.

In analyses relating *hypertension* with the risk of MCI in WHICAP, hypertension was related to a higher risk of all-cause and non-amnestic MCI, but not amnestic MCI. Non-amnestic MCI, as defined in our study, may be related in particular to cerebrovascular disease and vascular

dementia. Since hypertension is associated with a higher risk of cerebrovascular disease and vascular dementia, it seems reasonable that it must be related with the risk of non-amnestic MCI.

In **chapter 3**, I summarize studies exploring the relation between common variation in the genes encoding C-reactive protein and Metalloproteinase-3 with cerebral-small vessel disease and dementia.

C-reactive protein had been reported to be associated with various measures of vascular disease, but it remained unclear if these associations were in fact causal or rather caused by residual confounding. Cerebral small-vessel disease is associated with cognitive decline and dementia and may be an intermediate stage between vascular risk factors and dementia. In the Rotterdam Scan Study and the independent population of the 'Memory and Morbidity in Augsburg Elderly' (MEMO) Study, I related haplotypes, representing the common genetic variation in the gene encoding CRP, with measures of cerebral small-vessel disease. Variation in the CRP–gene was neither associated with white matter hyperintensities nor lacunar infarcts. This finding does not support the hypothesis that CRP plays a causal role in the pathogenesis of cerebral small-vessel disease, and suggests that previous findings might have been caused by residual confounding.

Findings of recent animal and post mortem studies suggested that matrix metalloproteinases (MMPs), a family of zinc- and calcium-dependent endopeptidases that are involved in the degradation of connective tissue and extracellular matrix, may be implicated in the pathogenesis of Alzheimer's disease. MMP-3 is directly involved in Aß degradation, and there is evidence that it has a reduced expression in Alzheimer's disease hippocampi, suggesting that it plays a role in selective neurodegeneration. MMP-3 is furthermore involved in the pathogenesis of atherosclerosis; common polymorphisms in the gene encoding MMP-3, in particular the 5A6A promotor polymorphism, have been repeatedly associated with the risk of atherosclerosis and cardiovascular disease, which in turn may be associated with the risk of cognitive impairment. I explored whether these associations can be replicated in a population-based sample, and related MMP-3 haplotypes with dementia and hippocampus volume in the Rotterdam Study and the Rotterdam Scan Study. There was no association of variation in the MMP-3 gene with dementia or hippocampus volume. This does not suggest that MMP-3 plays a causal role in the pathogenesis of dementia.

In **chapter 4**, I describe studies exploring the direct impact of cerebrovascular disease on the risk of cognitive impairment and dementia. In analyses relating stroke with cognitive performance over time in WHICAP, a history of stroke was related to a progressive decline in memory and abstract/visuospatial performance especially among men and those without an APOEε4 allele. In analyses relating incident stroke as a time-varying exposure with the risk of dementia and Alzheimer's disease in the Rotterdam Study, incident stroke was associated with a more than doubled risk of subsequent dementia, independent of pre-stroke cognitive performance and other potential risk factors for cognitive decline. This finding contradicts

previous studies reporting a higher risk of post-stroke dementia in persons with pre-stroke cognitive impairment compared with persons with normal cognition before stroke, and suggests that stroke increases the risk of subsequent dementia independent of pre-stroke level of cognitive function.

In **chapter 5**, I reflect on these findings in the context of current knowledge and potential methodological limitations, and give suggestions for future research.

In summary, various vascular risk factors were associated with the risk of dementia. Stroke more than doubled the risk of dementia independent from of pre-stroke level of cognitive function. In the context of current knowledge, the mechanisms underlying these associations remain unclear. The merge of modern genome science with population-based, epidemiological research may provide powerful tools to determine the pathways underlying vascular disease and cognitive impairment.

SUMMARY (in Dutch)

Dementie is één van de meest voorkomende neurodegeneratieve aandoeningen in de Westerse wereld en vormt een grote belasting voor de gezondheidszorg. De prevalentie van dementie neemt exponentieel toe met de leeftijd, van ongeveer 1% bij mensen tussen 60 en 64 jaar tot 60% bij mensen die ouder zijn dan 95 jaar.

Amyloidplaques en neurofibrillaire tangles worden gezien als de belangrijkste pathologische kenmerken van de ziekte van Alzheimer, wat het meest voorkomende dementiesyndroom is. Recente ontdekkingen hebben een gedetailleerd inzicht geboden in de moleculaire pathogenese van de veranderingen die optreden bij de ziekte van Alzheimer. Met het toenemen van de wetenschappelijke kennis komen we echter ook steeds meer tot het inzicht dat dementie een heterogene aandoening is waarbij vele factoren een rol spelen. Naast amyloidplaques en neurofibrillaire tangles schijnen – vooral in de pathogenese van de laatoptredende vorm van dementie ('late onset' of ouderdomsdementie) – andere factoren, met name cerebrovasculaire stoornissen, een rol te spelen.

Autopsiebevindingen tonen aan dat 15 tot 30 procent van het brein van oudere patiënten met 'late onset' dementie niet alleen meer amyloidplaques en neurofibrillaire tangles bevat, maar ook meer cerebrovasculaire veranderingen. In observationele studies zijn verbanden gevonden tussen verschillende vasculaire risicofactoren en cognitief functioneren of dementie. Ook is ontdekt dat beroertes het risico op dementie significant verhogen: de prevalentie van dementie na een beroerte wordt geschat op circa 30 procent.

Het grootste deel van deze bevindingen is gedaan in crossectionele studies, studies waarin de deelnemers maar korte tijd gevolgd werden, of in autopsiestudies. Andere bevindingen zijn gebaseerd op studies onder beroertepatiënten, bij wie het cognitief functioneren voorafgaand aan het optreden van de beroerte niet onderzocht kon worden. De lange preklinische fase van dementie, de pathologische diversiteit die ten grondslag ligt aan dementie, en de

onzekerheid of factoren die vaker in demente dan in niet-demente personen waargenomen worden werkelijk dementie veroorzaken of slechts tekenen zijn van veroudering, vereisen echter studies waarin deelnemers langer gevolgd worden om oorzaken en gevolgen van dementie te identificeren en van elkaar te kunnen onderscheiden.

Het doel van mijn proefschrift was om een diepgaander inzicht in vasculaire en genetische risicofactoren voor dementie te verkrijgen en om de invloed van beroertes op het risico op dementie op te helderen. Daarom heb ik gegevens van drie bevolkingsonderzoeken, die alle drie ontworpen zijn om oorzaken van neurodegeneratieve en cerebrovasculaire aandoeningen te bestuderen, geanalyseerd. Dit betreft de *Rotterdam Study* en de *Rotterdam Scan Study* van de afdeling Epidemiologie & Biostatistiek van het Erasmus Medisch Centrum Rotterdam, en het *Washington Heights Inwood Columbia Aging Project (WHICAP)* van het G.H. Sergievsky Center van de Columbia University in New York.

Nadat ik in **hoofdstuk 1** de wetenschappelijke achtergrond en het doel van dit proefschrift heb uitgelegd, geef ik in **hoofdstuk 2** een overzicht van de studies waarin de samenhang tussen diverse vasculaire risicofactoren (plasmalipidenspiegels, roken en hypertensie) en verschillende stadia van cognitieve achteruitgang tot en met dementie beschreven is.

In onderzoeken in de WHICAP-populatie, waarin ik de samenhang tussen *plasmalipidenspiegels* en cognitief functioneren en dementie onderzocht heb, waren verhoogde Low-Density Lipoprotein (LDL) en Non-High-Density Lipoprotein (non-HDL) cholestrolwaardes en verlaagde High-Density Lipoprotein (HDL) cholesterolwaardes met een verhoogd risico op dementie geassocieerd. Een verhoogde waarde van het totale cholesterol was daarentegen met een verlaagd risico op de ziekte van Alzheimer geassocieerd. Er was geen samenhang tussen plasmalipidenspiegels en verandering in cognitief functioneren of tussen plasmalipidenspiegels en *Mild Cognitive Impairment (milde cognitieve beperking; MCI)*, wat als overgangsstadium tussen normaal cognitief functioneren en dementie beschouwd wordt. Samengevat suggereren deze bevindingen dat dyslipidemie op oudere leeftijd geen grote rol speelt in de pathogenese van dementie.

Roken was in de Rotterdam Study in deelnemers zonder APOEε4-allel geassocieerd met een verhoogd risico op dementie en de ziekte van Alzheimer. In de WHICAP-studie was roken in deelnemers zonder APOEε4-allel geassocieerd met een snellere achteruitgang in cognitief functioneren, in het bijzonder met de achteruitgang van het geheugen. Er zijn twee mogelijke verklaringen voor deze bevindingen. Het is mogelijk dat het APOEε4-allel het dementierisico zo sterk verhoogt dat andere factoren, zoals bijvoorbeeld roken, niet bijdragen aan een verdere verhoging van het risico op dementie onder dragers van het APOEε4-allel. Het is echter ook mogelijk dat roken het risico op dementie door vasculaire mechanismen verhoogt, maar dat het in dragers van het APOEε4-allel deels beschermend werkt. Deze laatste verklaring wordt ondersteund door de waarneming dat Alzheimerpatiënten die drager zijn van minstens één

APOEε4-allel minder nicotinereceptoren en een lagere cholinacatyltransferase-activiteit hebben dan mensen zonder APOEε4-allel. In mijn onderzoek heb ik echter geen interactie tussen APOEε4-genotype en rookgewoonten gevonden. Bovendien hadden rokers die drager zijn van het APOEε4-allel een verhoogd, of hooguit hetzelfde, risico op dementie vergeleken met APOEε4-dragers die nooit gerookt hebben. Deze feiten ondersteunen eerder de hypothese dat roken daadwerkelijk schadelijk is, maar dat dit effect bij mensen die door het APOEε4-allel reeds een verhoogd risico hebben minder tot uiting komt.

Bij het analyseren van het verband tussen *hypertensie* en het risico op MCI in de WHICAP-studie, werd gevonden dat hypertensie gerelateerd was aan een hoger risico op MCI en MCI zonder amnesie, maar niet op MCI met amnesie. MCI zonder amnesie, zoals gedefiniëerd in onze studie is gerelateerd aan cerebrovasculaire ziekte en aan vasculaire dementie. Aangezien hypertensie geassocieerd is met een hoger risico op cerebrovasculaire ziekte en vasculaire dementie, lijkt het aannemelijk dat er een relatie bestaat tussen hypertensie en het risico op MCI zonder amnesie.

In **hoofdstuk 3** geef ik een overzicht van de studies die de relatie beschrijven tussen de variatie in genen die coderen voor C-reactief proteïne (CRP) en Metalloproteinase-3 en het onstaan van cerebrale microangiopathie en dementie.

In eerdere onderzoeken is CRP geassocieerd met verschillende kenmerken van vasculaire ziekte, maar het is nog steeds onduidelijk of deze associaties causaal van aard zijn of worden veroorzaakt door 'residual confounding' (andere factoren waar wij onze berekeningen niet voor konden corrigeren). Cerebrale microangiopathie is geassocieerd met cognitieve achteruitgang en dementie en zou mogelijk een overgangsstadium kunnen vormen tussen vasculaire risicofactoren en dementie. In de Rotterdam Scan Studie en in de onafhankelijke populatie van de "Memory and Morbidity in Augsburg Elderly" (MEMO) Studie, heb ik de invloed onderzocht van haplotypes die de gangbare genetische variatie vertegenwoordigen in het gen dat codeert voor CRP, op verschillende maten van cerebrovasculaire laesies. Variatie in het CRP-gen was niet met hyperdensiteit van de witte stof, noch met lacunaire infarcten geassocieerd. Dit resultaat is in tegenspraak met de hypothese dat CRP een causale rol speelt in de pathogenese van cererbale microangiopathie en lijkt erop te wijzen dat de resultaten van voorgaande studies mogelijk zijn te wijten aan 'residual confounding'.

Resultaten van recente post-mortem studies en studies in diermodellen geven aanwijzingen dat matrix-metalloproteinases (MMP's), een groep van zink-en calcium-afhankelijke endopeptidases die zijn betrokken bij de afbraak van bindweefsel en extracellulaire matrix, mogelijk een rol spelen bij de pathogenese van de ziekte van Alzheimer. MMP-3 is direct betrokken bij de degradatie van amyloid-beta en er zijn aanwijzingen dat er een gereduceerde expressie is van MMP-3 in hippocampi van Alzheimer-patiënten. Dit kan wijzen op een mogelijke rol van MMP-3 bij selectieve neurodegeneratie. MMP-3 is ook betrokken bij de

pathogenese van atherosclerose; er zijn herhaaldelijk associaties gevonden tussen gangbare polymorfismen in het gen dat codeert voor MMP-3 (in het bijzonder het 5A6A promotor polymorfisme) en het risico op atherosclerose en cardiovasculaire ziekte, die op hun beurt weer mogelijk zijn gerelateerd aan het risico op cognitieve achteruitgang. Ik heb bekeken of deze associaties kunnen worden gerepliceerd in een onderzoek in de algemene bevolking, en heb de relatie tussen MMP-3 haplotypes enerzijds en dementie en het hippocampusvolume anderzijds onderzocht. Er was geen associatie tussen variatie in het MMP-3 gen en dementie of het hippocampusvolume. Dit wijst erop dat MMP-3 geen causale rol speelt in de pathogenese van dementie.

In **hoofdstuk 4** geef ik een overzicht van studies waarin het directe effect is onderzocht van strokes (herseninfarcten en hersenbloedingen) op het risico op cognitieve achteruitgang en dementie. In longitudinale analyses van de associatie tussen stroke en cognitieve functie in het WHICAP-onderzoek werd aangetoond dat het gehad hebben van een stroke gerelateerd was met een progressieve achteruitgang van het geheugen en van de abstracte/visuospatiele functie, in het bijzonder in mannen en in mensen zonder het APOEε4-allel. In analyses van incidente strokes als tijdsafhankelijke blootstelling en het risico op dementie en de ziekte van Alzheimer in de Rotterdam Study bleek dat incidente stroke geassocieerd was met een meer dan verdubbeld risico op daarop volgende dementie. Dit was onafhankelijk van de cognitieve functie voorafgaand aan de stroke en van andere potentiële risicofactoren voor cognitieve achteruitgang. Dit wijst erop dat stroke het risico op post-stroke dementie verhoogt, onafhankelijk van de cognitieve functie voorafgaand aan de stroke. Hiermee spreken deze resultaten eerdere studies, die een hoger risico op post-stroke dementie vonden voor mensen met een lage pre-stroke cognitieve functie vergeleken met mensen met een normale pre-stroke cognitieve functie, tegen.

In **hoofdstuk 5** probeer ik deze resultaten te plaatsen in de context van het huidige kennisveld en de mogelijke methodologische knelpunten, en geef ik suggesties voor toekomstig onderzoek.

Samenvattend zijn er verschillende vasculaire risicofactoren geassocieerd met het risico op dementie. Stroke geeft een meer dan verdubbeld risico op dementie, onafhankelijk van het cognitieve niveau voorafgaand aan de stroke. De huidige staat van kennis in ogenschouw nemend, zijn de mechanismen die aan deze associaties ten grondslag liggen op dit moment nog niet bekend. De samensmelting van de moderne genetische wetenschap met het epidemiologische onderzoek in de algemene bevolking kan een krachtig instrument vormen dat eraan kan bijdragen om de mechanismen die ten grondslag liggen aan vasculaire ziekte en cognitieve achteruitgang te ontrafelen.

SUMMARY (in German)

Die Demenz ist eine der häufigsten neurodegenerativen Erkrankungen in der westlichen Welt und stellt eine der größten Belastungen für das Gesundheitssystem dar. Ihre Prävalenz steigt nahezu exponentiell mit zunehmendem Alter, von circa einem Prozent in 60-64 jährigen Personen auf bis zu 60 Prozent in Personen 95 Jahre oder älter.

Amyloidplaques und Neurofibrillen gelten als die neuropathologischen Hauptmerkmale des Morbus Alzheimer, welcher als häufigster Subtyp der Demenz angesehen wird. Fortschritt in der Demenzforschung hat in den letzten Jahren zu detailliertem Verständnis der molekularen Grundlagen dieser pathologischen Veränderungen geführt. Mit zunehmender wissenschaftlicher Kenntnis der Erkrankung steigen jedoch auch die Anzeichen, daß die Demenz eher eine heterogene und multifaktorielle Erkrankung ist. Neben Amyloidplaques und Neurofibrillen scheinen - vor allem in der Pathogenese der spätauftretenden Form der Demenz („late-onset Demenz", „Altersdemenz") - andere Faktoren, insbesondere zerebrovaskuläre Erkrankungen, eine Rolle zu spielen.

Autopsiestudien zeigen, daß 15 bis 35 Prozent der Gehirne älterer Menschen, welche zu Lebzeiten mit „late-onset Demenz" diagnostiziert wurden, nicht nur vermehrt Amyloidplaques und Neurofibrillen aufweisen, sondern auch erhebliche zerebrovaskuläre Veränderungen. Beobachtungsstudien haben Zusammenhänge zwischen verschiedenen vaskulären Risikofaktoren und kognitiver Leistungseinschränkung oder Demenz gezeigt. Weitere Studien haben berichtet, daß Schlaganfälle das Demenzrisiko signifikant erhöhen. Die Prävalenz von Demenz nach Schlaganfall in diesen Studien ist ca. 30 Prozent.

Ein Großteil dieser Beobachtungen stammt von Querschnittsstudien, Studien mit kurzer Follow-up-Zeit oder Autopsiestudien. Andere stammen von krankenhaus-basierten Studien an Schlaganfallpatienten, in denen die kognitive Leistungsfähigkeit vor Auftreten des Schlaganfalls nicht akkurat erfasst und in Betracht gezogen wurde. Die lange präklinische Phase

der Demenz, die pathologische Diversität, die den Demenzsymptomen unterliegt, und die Unsicherheit, ob Faktoren, welche häufiger in dementen als in nicht-dementen Personen beobachtet werden, wirklich die Erkrankung verursachen oder lediglich ko-exisitierende Veränderungen im Alter darstellen, erfordern jedoch Studien mit einer langen Follow-up-Zeit um Ursachen und Konsequenzen der Demenz zu identifizieren und differenzieren.

Ziel meiner Arbeit war es eine tiefere Einsicht in vaskuläre und genetische Risikofaktoren für Demenz zu erreichen, und den Einfluss von Schlaganfällen auf das Demenzrisiko zu klarifizieren. Zu diesem Zweck habe ich Daten dreier Bevölkerungsstudien analysiert, welche zur Exploration von Ursachen neurodegenerativer und zerebrovaskulärer Erkrankungen im Alter konzipiert worden waren: der „Rotterdam Study" und der „Rotterdam Scan Study" der Abteilung für Epidemiologie und Biostatistik des Erasmus Medical Center Rotterdam, und des „Washington Heights Inwood Columbia Aging Project (WHICAP)" des G.H. Sergievsky Center der Columbia University in New York.

Nachdem ich in **Kapitel 1 („chapter 1")** den wissenschaftlichen Hintergrund und das Ziel dieser Arbeit erläutere, fasse ich in **Kapitel 2 („chapter 2")** Studien zusammen, in welchen in den Zusammenhang von diversen vaskulären Risikofaktoren (Plasma-Lipidwerte, Rauchen, und Hypertension) mit verschiedenen Stadien kognitiver Leistungseinschränkung bis hin zur Demenz untersucht habe.

In Studien in der WHICAP-Population, in welchen ich den Zusammenhang zwischen *Plasma-Lipidwerten* und kognitiver Leistungsfähigkeit und Demenz untersucht habe, waren erhöhte Low-density Lipoprotein- und Non-high-density Lipoprotein Cholesterinwerte sowie erniedrigte High-density Lipoprotein Cholesterinwerte mit einem erhöhten Risiko von vaskulärer Demenz assoziiert. Gesteigerte Gesamtcholesterinwerte waren dagegen mit einem erniedrigten Risiko von M. Alzheimer verbunden. Es gab keinen Zusammenhang zwischen Plasma-Lipidwerten und kognitiver Leistungsfähigkeit über Zeit, oder Plasma-Lipidwerten und 'Mild Cognitive Impairment', welches als Übergangsstadium zwischen normaler kognitiver Funktion und Demenz angesehen wird. Insgesamt sprechen diese Ergebnisse nicht dafür, daß Dyslipidämie im Alter eine große Rolle in der Pathogenese der Demenz spielt.

Rauchen war in der Rotterdam Study in Personen ohne APOEε4-Allel mit einem erhöhten Risiko von Demenz und M. Alzheimer verbunden. In der WHICAP-Studie war es in Personen ohne APOEε4-Allel mit einem schnelleren Abbau kognitiver Leistungsfähigkeit über Zeit, insbesondere des Erinnerungsvermögens, assoziiert. Für diese Beobachtungen gibt es zwei potentielle Erklärungen. Es ist möglich, daß das APOEε4-Allel das Demenzrisiko so stark erhöht, daß andere Faktoren - wie z.B. das Rauchen- das Risiko in Trägern des APOEε4-Allels nicht weiter steigern. Es ist aber auch möglich, daß das Rauchen das Demenzrisiko durch vaskuläre Mechanismen erhöht, es aber in Trägern des APOEε4-Allels teilweise protektiv wirkt. Für diese Erklärung sprechen Beobachtungen, daß Personen mit M. Alzheimer, die Träger mindestens

eines APOEε4-Allels sind, weniger nikotinische Rezeptoren und eine niedrigere Aktivität von Cholinacetyltransferase aufweisen als Personen ohne APOEε4-Allel. In meinen Studien gab es keine Gen-Umwelt-Interaktionen zwischen APOEε4-Genotyp und Rauchgewohnheit. Zudem hatten Raucher, die Träger des APOEε4-Allels sind, ein -wenn überhaupt- erhöhtes Risiko. Diese Fakten unterstützen eher die Hypothese, daß Rauchen tatsächlich schädlich ist, aber daß dieser Effekt in Personen, die durch das APOEε4-Allel bereits ein erhöhtes Risiko haben, weniger zum Vorschein kommt.

Hypertension war in der WHICAP-Studie mit einem erhöhten Risiko von MCI insgesamt und nicht-amnestischem MCI, aber nicht mit amnestischem MCI assoziiert. Nicht-amnestisches MCI, wenn definiert wie in unserer Studie, ist mit einem erhöhten Risiko von zerebrovaskulären Erkrankungen sowie vaskulärer Demenz assoziiert. Da Hypertension das Risiko zerebrovaskulärer Erkrankungen und vaskulärer Demenz erhöht, erscheint es sinnvoll, daß es mit dem Risiko von nicht-amnestischem MCI assoziiert sein muss.

In **Kapitel 3 („chapter 3")**, beschreibe ich Studien, in welchen ich den Zusammenhang von Variation in Genen, die C-reaktives Protein (CRP) und Matrix-Metalloproteinase-3 (MMP-3) kodieren, mit zerebrovaskulären Läsionen und Demenz untersuche.

Im Vorfeld dieser Studien waren Zusammenhänge zwischen *CRP* und diversen vaskulären Veränderungen beschrieben worden. Es blieb jedoch unklar, ob diese Zusammenhänge tatsächlich kausal oder durch residuelle Störfaktoren, für die in den statistischen Analysen nicht kontrolliert werden konnte, bedingt waren.

Arteriosklerose kleiner Hirngefässe ('cerebral small-vessel disease') ist mit einem erhöhten Risiko von eingeschränker kognitiver Leistungsfähigkeit und Demenz verbunden, und stellt möglicherweise ein Übergangsstadium zwischen vaskulären Risikofaktoren und Demenz dar. Ich habe in der Rotterdam Scan Study und der unabhängigen Population der 'Memory and Morbidity in Augsburg Elderly (MEMO)-Studie' den Einfluss von Haplotypen, welche die gesamte gängige Variation im CRP-Gen repräsentieren, auf verschiedene Ausprägungen von 'cerebral small-vessel disease' untersucht. Variation im CRP-kodierenden Gen war in keiner der beiden Studienpopulationen mit Läsionen der weissen Hirnsubstanz oder lakunären Hirninfarkten assoziiert. Diese Beobachtung spricht nicht dafür, daß CRP eine kausale Rolle in der Pathogenese zerebrovaskulärer Läsionen spielt. Sie deutet vielmehr an, daß frühere Bebachtungen eines Zusammenhangs zwischen CRP-Plasmawerten und vaskulären Veränderungen eher durch den Einfluss residueller Störfaktoren verursacht wurden.

Beobachtungen früherer Tier- und post-mortem-Studien haben angedeutet, daß *Matrix-Metalloproteinasen (MMPs),* eine Familie zink- und calciumabhängier Endopeptidasen, die in die Degradation von Bindegewebe und extrazellulärer Matrix involviert sind, eine Rolle in der Pathogenese von M. Alzheimer spielen könnten. MMP-3 ist unmittelbar an der Degradation von Beta-Amyloid-Protein beteiligt, und es gibt Hinweise, daß es eine reduzierte Expression

in Alzheimer-Hipocampi hat. Dies könnte auf eine Rolle von MMP-3 in selektiver Neurodegeneration hindeuten. MMP-3 scheint darüber hinaus an der Pathogenese von Arteriosklerose beteiligt zu sein. Gängige Polymorphismen im MMP-3-kodieren Gen, insbesondere der 5A6A-Promotor-Polymorphismus, sind wiederholt im Zusammenhang mit einem erhöhten Risiko von Arteriosklerose und koronarer Herzerkrankung beobachtet worden. Diese sind widerum mit einem gesteigerten Risiko von kognitiver Leistungseinschränkung assoziiert. Ich habe in der Rotterdam Study und der Rotterdam Scan Study die Assoziation zwischen MMP-3-Haplotypen und Demenz und Hippocampusvolumen erforscht. Variation im MMP-3-Gen war weder mit einem erhöhten Risiko von Demenz noch mit Veränderungen im Hippocampusvolumen verbunden.

In **Kapitel 4 („chapter 4")** beschreibe ich Studien, in welchen ich den direkten Einfluss zerebrovaskulärer Erkrankungen auf das Risiko von kognitiver Leistungseinschränkung und Demenz untersucht habe. In der WHICAP-Studie, in der ich den Zusammenhang zwischen Schlaganfällen und kognitiver Funktion über Zeit untersucht habe, war eine positive Schlaganfallanamnese mit einem schnelleren Abbau in Erinnerungsvermögen und abstraktem/räumlichem Denken assoziiert, insbesondere bei Männern und Personen ohne APOE-ε4-Allel. In der Rotterdam Study, in der ich den Zusammenhang zwischen neuaufgetretenem Schlaganfall (als zeit-variierende Exposition) mit dem Risiko von Demenz und M. Alzheimer untersucht habe, waren neuaufgetretene Schlaganfälle mit einem mehr als doppelten Risiko von Demenz verbunden. Dieser Zusammenhang war unbeeinflusst von der Höhe kognitiver Leistungsfähigkeit vor Schlaganfall und anderen potentiellen Risikofaktoren für Demenz. Dieses Ergebnis widerspricht früheren Studien, welche ein höheres Risiko von Demenz in Personen mit eingeschränkter kognitiver Funktion vor Schlaganfall als in Personen mit normaler kognitiver Funktion vor Schlaganfall beschrieben haben. Es deutet vielmehr darauf hin, daß Schlaganfälle das Risiko von Demenz unabhängig von der kognitiven Leistungsfähigkeit vor Schlaganfall erhöhen.

In **Kapitel 5 („chapter 5")** diskutiere ich alle Ergebnisse dieser Arbeit im Kontext aktuellen Wissens und potentieller methodologischer Limitationen. Zudem gebe ich Anregungen für zukünftige Forschungsschwerpunkte.

In dieser Arbeit waren verschiedene vaskuläre Risikofaktoren mit einem erhöhten Risiko von Demenz assoziiert. Neuaufgetretene Schlaganfälle steigerten das Risiko von Demenz um mehr als das Doppelte. Im Kontext aktuellen Wissens bleiben die Pathomechanismen, die diesen Zusammenhängen unterliegen, unklar. Die Verschmelzung moderner Genomwissenschaft mit bevölkerungsbasierter, epidemiologischer Forschung könnte kraftvolle Instrumente zur Identifizierung dieser Mechanismen bieten.

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Christiane Reitz was born on October 10, 1975 in Lüdenscheid, Germany. After attending secondary school at Bergstadt-Gymnasium Lüdenscheid, she entered medical school at Westfälische Wilhelms-Universität Münster in October 1994. During her medical education, she coordinated a research project at the Department of Dermatology exploring the therapeutic effect of Clindamycin on the course of Staphylodermia follicularis profunda. For this work she obtained a doctoral title in 2001, the same year she graduated from medical school. From June 2002 through December 2004 she did a fellowship at the Gertrude H. Sergievsky Center at Columbia University in New York City, USA (Prof. Dr. Richard Mayeux), where she performed large parts of the work described in this thesis. To expand her education to the field of genetic epidemiology, she entered in August 2004 the NIHES degree program in genetic epidemiology at Erasmus MC, Rotterdam (program director: Prof. Dr. Cornelia van Duijn), from which she graduated in June 2006. From January 2005 through February 2006 she worked at Westfälische Wilhelms-Universität Münster, Germany, and continued to pursue her research in the field of cognitive and cerebrovascular disorders by collaboration with Prof. Dr. Klaus Berger from the Institute of Epidemiology and Social Medicine and Prof. Monika Stoll from the Leibniz-Institute for Arteriosclerosis Research. In November 2005, she was awarded a 1-year personal research grant by the Ministry of Innovation, Science, Research and Technology of the State of North Rhine-Westphalia, Germany. Since March 2006 she works with Prof. Dr. Monique M.B. Breteler in the Neuroepidemiology Unit at the Department of Epidemiology & Biostatistics (Prof. Dr. A. Hofman) at Erasmus Medical Center, Rotterdam.