Determinants and Outcomes of Structural Brain Changes

Mohammad Arfan Ikram

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For my family

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Part 1

Chapter 1

Introduction

Of all the organs in the human body, the brain is often considered as the most mysterious and least understood, yet most indispensable organ. Both the development in early years and degeneration in late life are poorly understood. Neuro-degeneration in late life often causes clinical disorders, such as dementia, cognitive decline, stroke, and depression. From a public health perspective, these neurodegenerative disorders put a massive burden on health resources and health care: not only are these disorders already highly frequent, but with the increasingly older population their prevalence and incidence are only expected to further increase in prevalence and incidence.[1] In order to prevent these diseases knowledge of their etiology is an essential first step. Research using neuro-imaging and genetics has played a central role in finding etiologic markers of neurodegenerative disease.

Using MRI various studies have identified in vivo imaging markers of neurodegenerative diseases.[2-15] Such markers include focal structural brain changes, such as hippocampal atrophy, white matter lesions and lacunar infarcts, and are usually rated visually on MRI scans. Hippocampal atrophy is considered a marker of Alzheimer pathology and indicates an increased risk of dementia, even in cognitively normal persons. White matter lesions and lacunar infarcts are markers of vascular brain disease and have been implicated in the etiology of dementia, stroke, and depression. However, apart from these focal brain changes, generalized brain changes are also frequently seen. It has been suggested that brain atrophy may be an important marker of neurodegenerative diseases. However, research on whole brain atrophy has been limited, partly because appropriate quantification methods were lacking. The difficulty lay not only in obtaining an accurate and reproducible measure for

brain atrophy, but also in distinguishing gray matter atrophy from white matter atrophy and in separating the different cerebral lobes. Advances in MRI technology and image processing have recently led to the ability to automatically quantify volumes of various brain tissues and structures. As a result, we are now able to investigate generalized structural brain changes, such as whole brain atrophy and lobar atrophy. Moreover, exact quantification of focal brain changes increases the power to detect more subtle associations, which could not be detected previously using qualitative or semi-quantitative rating scales.

Until recently, the quest for finding genes and genetic markers of neurological diseases has been disappointing. Although linkage studies and candidate gene studies implicated various genetic markers in the etiology of neurodegenerative diseases, replication studies usually failed to confirm these associations. Only the *APOE* gene in the etiology of late-onset Alzheimer's disease can be considered as a robust finding.[16-18] One reason for this dearth of genetic markers for neurodegenerative diseases is that thus far genetic research was restricted to investigating single or few markers. These markers had to be selected a priori from certain genomic loci based on available (and often incomplete) biological knowledge of the disease. Only recently has it become possible to investigate genetic markers on a large scale across the whole genome. These hypothesis-free 'genome-wide association studies' have uncovered several previously unknown genes for various complex diseases, such as diabetes, heart disease, and auto-immune disorders.[19] Likewise, identifying novel genes involved in neurological disorders using the genome-wide approach is now possible and its results eagerly anticipated.

The aim of this thesis is to investigate determinants – both non-genetic and genetic – and clinical outcomes of structural brain changes on MRI. The focus will be on markers across the whole brain and whole genome and their relationship with neurodegenerative diseases. The research is embedded within the epidemiological framework of the Rotterdam Study and Rotterdam Scan Study, both large prospective population-based cohort studies with more than a decade of continuous follow-up. The aim of these studies is to investigate genetic and non-genetic determinants of chronic diseases in the elderly, including neurological diseases. In the Rotterdam Scan Study an additional focus is on structural brain changes as seen on MRI.

In the following two parts of this thesis I investigate determinants and outcomes of brain changes on MRI. **Part 2** embarks by presenting descriptives of structural brain changes: in **chapter 2.1.1** normative, expected values of brain tissue volumes in the elderly population are presented together with their association with known cardiovascular risk factors. **Chapter 2.1.2** describes pathologic changes in the brain that are unexpected and therefore can be considered as incidental findings. **Part 2** continues by investigating novel risk fac-

tors and risk indicators and their association with brain changes. These novel determinants are kidney function (**chapter 2.2**), unrecognized myocardial infarction (**chapter 2.3**), cerebral blood flow (**chapter 2.4**) and retinal vessels (**chapter 2.5**). **Part 3** of this thesis is dedicated to the relationship of brain changes with cognition and dementia (**chapter 3.1**), depression (**chapter 3.2**), and all-cause and cardiovascular mortality (**chapter 3.3**). In **Part 4** of the thesis, two genetic studies are presented that apply the novel genome-wide association approach. **Chapter 4.1** presents results from genome-wide association studies on clinical stroke and subclinical stroke, respectively. The following chapter (**4.2**) is a replication study of a gene that was previously identified in a genome-wide association study. In **Part 5**, I round up the main findings and reflect on these from a broader perspective regarding brain research. I also tentatively give directions for future research.

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Part 2

Chapter 2.1

Structural Brain Changes

Chapter 2.1.1

Brain Tissue Volumes in the General Elderly Population. The Rotterdam Scan Study

M. Arfan Ikram, Henri A. Vrooman, Meike W. Vernooij, Fedde van der Lijn, Albert Hofman, Aad van der Lugt, Wiro J. Niessen, Monique M.B. Breteler

ABSTRACT

We investigated how volumes of cerebrospinal fluid (CSF), grey matter (GM) and white matter (WM) varied with age, sex, small vessel disease and cardiovascular risk factors in the Rotterdam Scan Study. Participants (n=490; 60-90 years) were non-demented and 51.0% had hypertension, 4.9% had diabetes mellitus, 17.8% were current smoker and 54.0% were former smoker. We segmented brain MR-images into GM, normal WM, white matter lesion (WML) and CSF. Brain infarcts were rated visually. Volumes were expressed as percentage of intra-cranial volume. With increasing age, volumes of total brain, normal WM and total WM decreased; that of GM remained unchanged; and that of WML increased, in both men and women. Excluding persons with infarcts did not alter these results. Persons with larger load of small vessel disease had smaller brain volume, especially normal WM volume. Diastolic blood pressure, diabetes mellitus and current smoking were also related to smaller brain volume. In the elderly, higher age, small vessel disease and cardiovascular risk factors are associated with smaller brain volume, especially WM volume.

INTRODUCTION

During lifespan the human brain undergoes structural changes. Using quantification of brain structures on magnetic resonance imaging (MRI) several studies have investigated changes in the volumes of cerebrospinal fluid (CSF), grey matter (GM) and white matter (WM) over age ranges from childhood to early senescence.[1-9] GM is thought to decrease steadily after adolescence, whereas WM shows a peak around age 40 and decreases thereafter. Linear extrapolation to older age remains tentative, because of possible nonlinear and non-monotonous effects.[10] Moreover, in the elderly cerebral small vessel disease and neurodegenerative processes are often present[11] and can affect brain tissue volumes,[12] which is not always taken into account. Only a few studies have reported the effects of cerebral small vessel disease on brain tissues in the elderly: DeCarli et al. showed that presence of lacunar brain infarcts was associated with smaller whole brain volume.[13] Guttman et al. and Jernigan et al. reported that a decrease in normal WM not only coincides with an increase in white matter lesions (WML), but also reflects atrophy of total WM.[12, 14] These studies either did not investigate the various brain tissues separately or were performed in selected participants.

Therefore, there is need for a large population-based study in elderly persons investigating various brain tissues separately. Using automated image analysis of MRI data, we quantified brain tissue volumes in a population-based study of elderly and investigated how these volumes were related to age, sex, markers of small vessel disease, and cardiovascular risk factors.

METHODS

Participants

This study is based on the Rotterdam Scan Study, a large population-based cohort study in the Netherlands that aims to study the etiology and natural history of age-related brain changes in the elderly.[11] The study was approved by the medical ethics committee of the Erasmus Medical Center, Rotterdam. In 1995-1996, we randomly invited participants, aged 60 to 90 years, stratified by sex and 5-year age strata from two on-going population-based studies to participate in the Rotterdam Scan Study.[15, 16] Participants who were demented, were excluded based on a stepwise approach as used in the Rotterdam Study.[17] Briefly, participants were screened with the Mini-Mental State Examination (MMSE) and Geriatric Mental State Schedule (GMS). Screen-positives (MMSE < 26 or GMS > 0) underwent further testing using the Cambridge examination for mental disorders in the elderly (CAMDEX). Finally, a panel consisting of a neurologist, neuropsychologist and research physician made the diagnosis based on internationally accepted criteria. After exclusion of individuals who were demented or had MRI contraindications 1,077 participants gave their written informed consent to participate in the study, which included MR brain imaging. The present study is restricted to persons (n=563), who underwent an additional high-resolution MR sequence.

MRI acquisition

MR brain imaging was performed on a 1.5-Tesla MRI System (VISION MR, Siemens AG, Erlangen, Germany). The protocol included three axial scans, i.e. T1-weighted (TR=700, TE=14, NEX=1, matrix 192x256, FOV=256x256, flip angle=80°, 20 slices), proton-density (PD) weighted (TR=2200, TE=20, NEX=1, matrix=192x256, FOV=256x256, flip angle=80°, 20 slices) and T2-weighted (TR=2200, TE=80, NEX=1, matrix=192x256, FOV=256x256, flip angle=80°, 20 slices). Slice thickness was 5 mm with an interslice gap of 20%. Furthermore, a high-resolution, Inversion-Recovery double contrast, 3-D half-Fourier acquisition single-shot turbo spin echo (HASTE) sequence was included (inversion time=4400ms, TR=2800, matrix=192x256, FOV=256x256, 128 contiguous sagittal slices of 1.25 mm). The interpolated voxel dimensions were 1x1x1.25mm^3. Two HASTE modules were sequentially acquired after the inversion pulse (effective TE of 29 ms and 440 ms). Each HASTE module combined nonselective radio frequency excitations to provide a short interecho spacing of 3.9 ms. We used the PD-weighted, T2-weighted and first HASTE module (HASTE-Odd) for automated brain tissue classification.

Among the 563 participants, 52 developed claustrophobia during MRI acquisition. Complete data were available in 511 persons. Twenty-one datasets were unusable due to excessive ghosting artifacts (n=5), scanning outside the range of coil sensitivity (n=10), or other reasons (n=6), leaving a total of 490 participants in our present study.

MRI analysis

Data were transferred and stored onto a Linux Workstation. Preprocessing steps and the tissue classification algorithm have been described previously.[18] In summary, preprocessing included registration of the T2 and PD scans to the HASTE-Odd scan, non-uniformity correction and scaling of the intensities to zero mean and unit variance (tools obtained from the Brain Imaging Center, Montreal; www.bic.mni.mcgill.ca). Two trained neuro-imagers independently performed manual labeling of twelve scans into five tissue classes (GM, normal WM, CSF, WML and background) to create a three-dimensional training feature-space. Using these training data we subsequently classified scans with k-nearest-neighbor (kNN) tissue classification. kNN has been successfully applied in neuroimaging studies.[2, 19] In order to minimize any misclassification of partial volume voxels as WML around cortical GM, we registered to each brain a manually created mask, within which voxels could be classified as WML.

To remove non-brain tissue, we used a validated, non-rigid transformation based on free-form deformations.[20] A fast implementation of this algorithm (Elastix) was used to transform one template scan to each brain.[21] In this template all non-cerebral tissues, including e.g. eyes, skull and skin, were manually masked. Because the PD and T2 scans comprised only 20 slices, the cerebellum and brainstem were not always completely covered. Therefore, we also masked all infratentorial tissue (including the cerebellum) from our template scan. To control for potential sex differences we used a different template for men and women. The templates were chosen from our cohort as the scans nearest to the sex-specific mean, which was obtained based on previously conducted visual rating of all scans for cortical atrophy, subcortical atrophy and hippocampal volume.[11] Figure 1 shows a typical classification result.

Finally, two trained neuro-imagers, blinded for clinical information, independently went through all scans on a slice-by-slice basis to verify the classification result. If needed, voxels that were misclassified were manually reclassified appropriately. This was needed in only 45 scans, mostly due to misclassification because of slight motion artifacts.

We validated the classification and transformation algorithms using a leave-one-out strategy within the twelve manual classifications.[18] We used similarity indices and intra-class correlation coefficients (ICC) as validation measures. The similarity index is a measure ranging from 0 to 1 indicating the amount of overlap between two segmentations.[22] Values above 0.7 indicate excellent overlap.[22] The averaged similarity indices between the automated classification and manual labeling were 0.91 for CSF, 0.92 for GM, 0.93 for normal WM and 0.63 for WML. The similarity indices between the two neuro-imagers were 0.91 for CSF, 0.90 for GM, 0.91 for normal WM and 0.79 for WML. The averaged ICC between the automated classification and manual labeling was 0.89 for CSF, 0.94 for GM, 0.80 for normal WM and 0.84 for WML. The ICCs between the two neuro-imagers were slightly higher (range 0.84-0.96). These numbers indicate very good to excellent agreement and overlap.

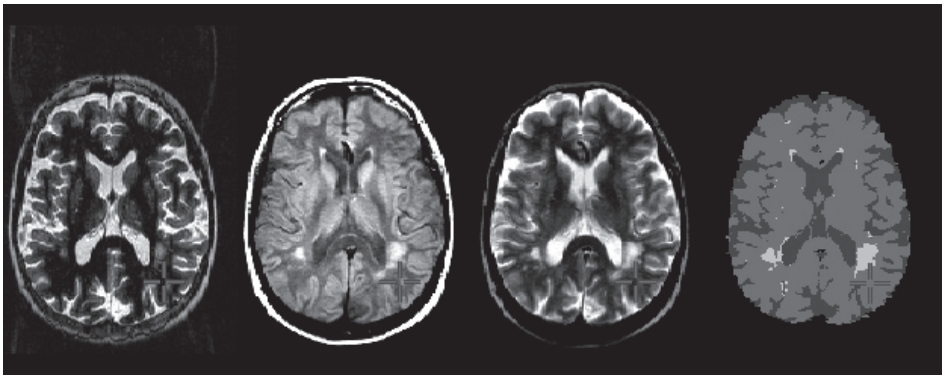

Figure 1. From left to right: HASTE-Odd scan, Proton-density weighted scan, T2 weighted scan, and result after tissue classification and removal of non-brain tissues (no manual reclassification performed; blue: cerebrospinal fluid; green: grey matter; red: white matter; white: white matter lesion). See this figure in color in the Appendix.

For the brain templates used to remove non-brain tissue, the ICCs compared to manual tracings were 0.97 for the male mask and 0.99 for the female mask. To assess the impact of different brain masks for men and women we applied the male template to 100 women and the female template to 100 men. This had only marginal effect on our results (ICC>0.98 for all tissue classes).

Brain infarcts were rated visually as focal hyperintensities on T2-weighted images, 3 mm in size or larger. Hyperintensities in WM also had to have corresponding prominent hypointensities on T1-weighted images. Proton-density scans were used to distinguish infarcts from dilated perivascular spaces. Infarcts were scored as lacunar or cortical. Persons with both lacunar and cortical infarcts were included in the group with cortical infarcts. Intrarater agreement for detection of infarcts was good (κ=0.80).[11]

Other measures

Information on current health status was collected by interview and physical examination.[11] Level of education was assessed during the interview and we classified persons into those with primary education only or those with education beyond primary level. Participants were also asked about their smoking habits and they were classified into one of three categories: current smoker, former smoker, or never smoker. At the research center, sitting blood pressure was measured twice at the right arm with a random-zero sphygmomanometer. The average of the two values measured at one occasion was used. Hypertension was defined as one of the following: a systolic blood pressure of 160 mm Hg or higher, or a diastolic blood pressure of 100 mm Hg or higher, or current use of blood pressure lowering drugs. This corresponds to grades 2 and 3 according to the 1999 World Health Organization guidelines.[23] Diabetes mellitus was defined as non-fasting serum glucose level exceeding

11.1 mmol/l or the use of oral blood glucose lowering drugs or insulin. All persons with diabetes mellitus had diabetes type 2.

Statistical analysis

Summing all voxels (1 voxel = 1.25mm^3) of a certain tissue across the whole brain yielded volumes in milliliters. To correct for individual head size, we expressed all volumes as percentages of intra-cranial volume, which was the sum of GM, total WM and CSF. We defined total WM volume as the sum of normal WM and WML. Brain volume (BV) was the sum of GM and total WM. WML volume was further natural log (ln) transformed because of leftward skewness of the untransformed measure.

We investigated differences between men and women with AN(C)OVA and the relationship between age and tissue volumes with linear regression. We also assessed differences between sexes in the effect of age. For WM, a non-linear relation with age has been reported.[10] Therefore, we also added a quadratic term of age to the linear model. To assess whether our results could be confounded by tissue loss due to infarcts, we repeated the same analyses excluding persons with any brain infarct. Differences in tissue volumes in persons without brain infarcts, with lacunar infarcts and with cortical infarcts were investigated using AN(C)OVA. With linear regression models we investigated the relationship between WML (per standard deviation (SD)) and brain tissues. All analyses were performed adjusting for age, sex (if applicable), and additionally for education, and cardiovascular risk factors.

Finally, with linear regression models we investigated how education, current smoking, former smoking, hypertension, systolic blood pressure, diastolic blood pressure, and diabetes mellitus were related to brain tissue volumes and whether their effects were different between men and women. Initially, we investigated the effect of a risk factor only adjusting for age and

Table 1. Characteristics of the study population, stratified by sex.

	Total n=490	Men n=241	Women n=249	p-value*
Age, yr [a]	73.4 ± 7.9	73.7 ± 7.7	73.1 ± 8.0	0.368
Height, cm	168 ± 9	174 ± 6	162 ± 7	<0.001
Intra-cranial volume, ml	1128 ± 116	1201 ± 97	1057 ± 84	<0.001
Cortical brain infarct [b]	25 (5.1)	17 (7.1)	8 (3.2)	0.12
Lacunar brain infarct	112 (22.9)	50 (20.7)	62 (24.9)	0.24
Hypertension	250 (51.0)	128 (53.1)	122 (49.0)	0.47
Diabetes mellitus	24 (4.9)	18 (7.5)	6 (2.4)	0.01
Current smoker	87 (17.8)	48 (20.0)	39 (15.7)	<0.001
Former smoker	264 (54.0)	194 (72.5)	90 (36.1)	<0.001
Primary education only	149 (30)	54 (22)	95 (38)	<0.001

[a] values are means ± standard deviation for continuous variables

[b] values are numbers (percentages) for dichotomous variables

* p-value for age-adjusted differences between men and women.

Table 2. Means and differences per year increase in age of brain tissue volumes.

	Men			Women				
	Mean volume [a]	Difference in volume (95% CI) [b]		Mean volume [a]	Difference in volume (95% CI) [b]		p-value sex difference*	p-value interaction**
Brain volume	77.0 ± 3.6	-0.31	(-0.36;-0.27)	77.8 ± 3.7	-0.32	(-0.36;-0.28)	0.006	0.75
Grey matter	45.6 ± 3.9	0.06	(-0.01;0.12)	47.5 ± 4.0	0.02	(-0.04;0.09)	<0.001	0.48
Normal white matter	30.3 ± 6.4	-0.42	(-0.52;-0.34)	28.7 ± 6.3	-0.43	(-0.51;-0.35)	<0.001	0.90
White matter lesions [c]	-0.61 ± 1.2	0.066	(0.046;0.085)	-0.06 ± 1.19	0.061	(0.044;0.078)	<0.001	0.71
Total white matter	31.3 ± 5.8	-0.37	(-0.45;-0.28)	30.3 ± 5.6	-0.34	(-0.42;-0.27)	0.006	0.70

Volumes are expressed as percentage of intra-cranial volume.

[a] values are means ± standard deviation

[b] values are differences in volume per year increase in age, with 95% confidence interval

[c] natural log transformed

* p-value for age-adjusted differences in means between sexes ** p-value for interaction between age and sex

sex. Then, using multivariable modeling we also adjusted for the other risk factors. Subsequently, we investigated any interaction of education with the cardiovascular risk factors and interactions between cardiovascular risk factors.

Results are presented with 95% confidence intervals (CI).

RESULTS

Table 1 shows the characteristics of the study population. We did not find a significant association between age and intra-cranial volume (sex-adjusted difference per year increase: -0.76 (95%CI -1.79;0.26)). This relation was further attenuated after adjusting for height (-0.15 (95%CI -1.19;0.89)), suggesting that this may reflect a secular trend. Brain infarcts were present in 137 persons (28%), of whom 112 had lacunar infarcts only.

When expressed as percentages of intracranial volume, men had a significantly smaller BV and larger volume of total and normal WM than women, whereas women had more GM and WML than men (table 2). Table 2 also shows the effect of age on the various brain tissues, in strata of sex. Figure 2 shows corresponding scatterplots for these relationships. BV and volumes of both normal WM and total WM were smaller with increasing age. No association with age was found for GM. WML showed a significant increase with age. Effect of age on tissue volumes was not different between men and women (table 2). Adding a quadratic term of age did not improve the linear model for total WM (p=0.22 for men and p=0.19 for women). These results did not change after additional adjustment for education, diabetes mellitus, hypertension,

Table 3. Association of education and cardiovascular determinants with intra-cranial volume and brain tissue volumes.

	ICV [a]	Brain volume [b]	GM [b]	Normal WM [b]	Total WM [b]
Primary education only (yes vs no)	-19.3 (-37.3;-1.4)	0.00 (-0.53;0.53)	-0.07 (-0.86;0.72)	-0.07 (-1.13;0.99)	0.07 (-0.92;1.06)
Hypertension (yes vs no)	11.2 (-5.3;27.7)	-0.43 (-0.91;0.06)	-0.20 (-0.92;0.52)	-0.46 (-1.43;0.52)	-0.23 (-1.14;0.68)
Systolic blood pressure (per SD)	-6.7 (-15.0;1.6)	-0.04 (-0.29;0.21)	0.26 (-0.11;0.62)	-0.44 (-0.93;0.05)	-0.30 (-0.75;0.16)
Diastolic blood pressure (per SD)	-0.4 (-8.5;7.7)	-0.27 (-0.50;-0.03)	0.35 (-0.01;0.70)	-0.77 (-1.25;-0.30)	-0.61 (-1.05;-0.17)
Diabetes mellitus (yes vs no)	27.8 (-10.0;65.6)	-1.53 (-2.64;-0.43)	-1.55 (-3.20;0.10)	0.40 (-1.83;2.62)	0.01 (-2.06;2.09)
Smoking					
Former vs never	9.4 (-11.8;30.6)	-0.40 (-1.02;0.22)	-0.49 (-1.42;0.44)	0.33 (-0.92;1.58)	0.09 (-1.07;1.25)
Current vs never	5.6 (-26.0;18.4)	-0.84 (-1.60;-0.08)	0.14 (-0.99;1.28)	-0.66 (-2.18;0.87)	-0.98 (-2.41;0.44)

Values are difference in tissue volumes (95% confidence interval), adjusted for age and sex. ICV intra-cranial volume, GM grey matter, WM white matter, SD standard deviation.

[a] expressed in milliliters

[b] expressed as percentage of intra-cranial volume

systolic and diastolic blood pressure, and smoking or when persons with any infarct on MRI were excluded (data not shown).

Figure 3 shows mean tissue volumes in persons grouped according to presence of infarcts. Persons with a lacunar brain infarct had smaller BV and smaller volumes of normal and total WM. No significant differences were found for GM. Similarly, persons with more WML had smaller BV (age and sex adjusted difference in BV per SD increase in ln WML was -0.40% (95%CI -0.66;-0.13)). The smaller BV was particularly caused by smaller total WM volume (age and sex-adjusted difference per SD increase in ln WML was -0.98% (95%CI -1.47;-0.49)). GM did not decrease with increasing WML (age and sex-adjusted difference per SD increase in ln WML was 0.58% (95%CI 0.19;0.98)). The associations between markers of small vessel disease and brain tissue volumes did not change after we additionally adjusted for education and cardiovascular risk factors.

Finally, table 3 shows how education and cardiovascular risk factors were related to brain tissue volumes, adjusting for age and sex. Higher education was related to larger ICV, but not to BV. Diastolic blood pressure, current smoking and diabetes mellitus were all related to smaller BV. Furthermore, diabetes mellitus was more associated with GM than total WM, although not significantly, whereas diastolic blood pressure and current smoking were more related to total WM than GM. Investigating the effects of cardiovascular risk factors, while additionally adjusting for the other risk factors, yielded similar results. These effects did not differ between sexes. Also, we did not

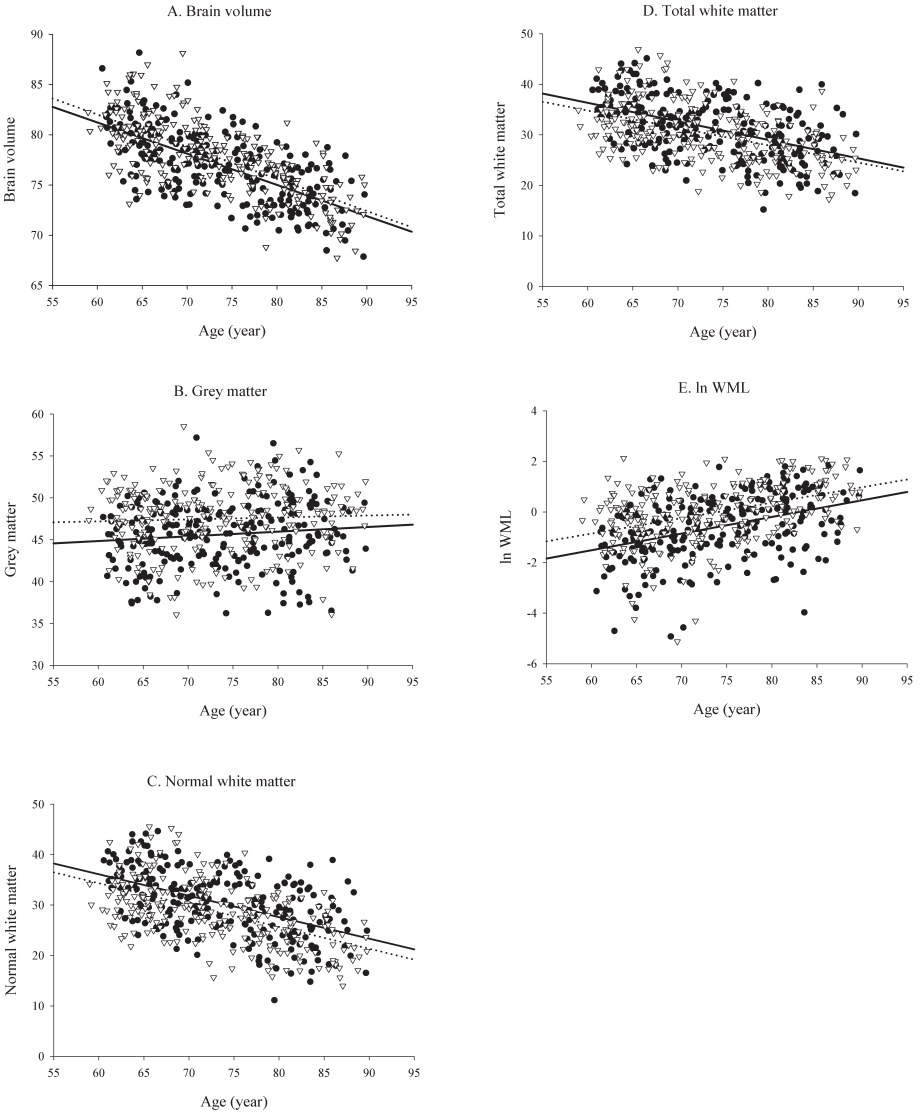

Figure 2. Scatterplots of tissue volumes against age, segregated by sex.
A. brain volume; B. grey matter; C. normal white matter; D. total white matter; E. white matter lesions (natural log transformed). Regression lines for linear fit are shown. Volumes are expressed as percentage of intra-cranial volume. Women: open triangles and dotted line. Men: closed circles and solid line.

find any significant interaction of education with age, sex or these cardiovascular risk factors nor between cardiovascular risk factors.

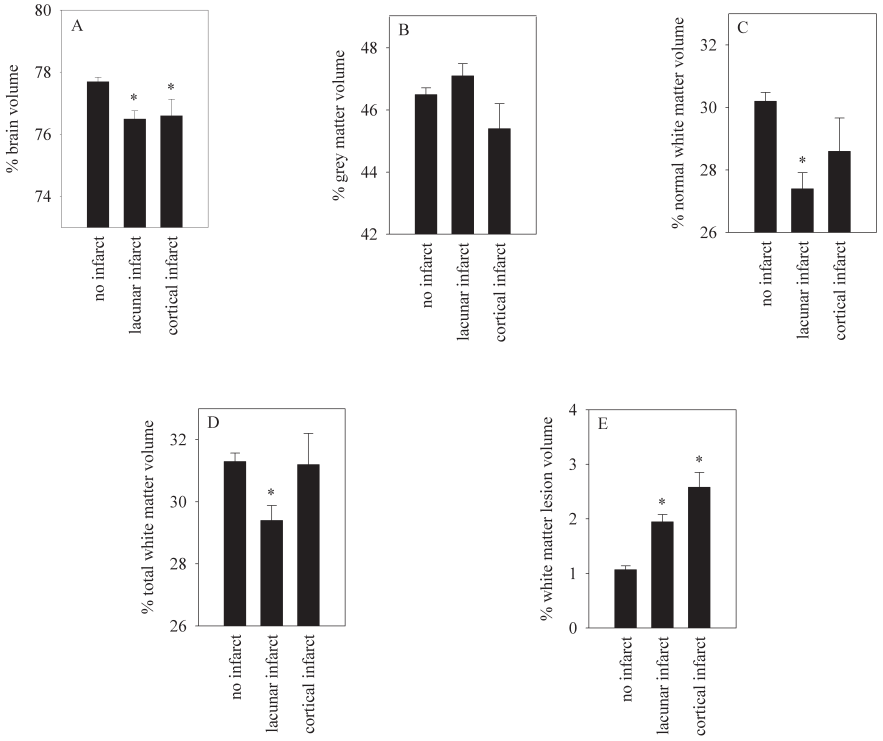

Figure 3. Age- and sex-adjusted mean tissue volumes in persons without brain infarcts (n=353), with lacunar infarcts (n=112) and cortical infarcts (n=25).

A. brain volume; B. grey matter; C. normal white matter; D. total white matter; E. white matter lesions.

Volumes are expressed as percentage of intra-cranial volume. Bars represent means; lines represent standard errors. * Significantly different from persons without any infarct ($p<0.05$).

DISCUSSION

Our study shows that in people aged 60 years and older, men had proportionally smaller BV, but more total WM than women, whereas women had more GM. Women also had more WML than men. Furthermore, increasing age was associated with smaller BV. This was particularly due to smaller volume of total WM and not GM. We found no difference between the sexes in the effect of age on brain tissue volumes. Finally, cerebral small vessel disease, current but not past smoking, diastolic blood pressure and diabetes mellitus were all related to smaller BV.

A major strength of our study is the population-based setting. This approach gives a good indication of brain changes in the general population, and with follow-up will allow for investigating determinants and consequences of brain changes related to cognitive impairment and dementia. Other strengths are the large sample size of elderly persons and the quantification of all brain tissues, as well as WML. Moreover, we used highly reliable

automated quantification methods. Limitations of our study include the cross-sectional study-design, which limits our understanding of intra-individual brain changes. However, comparable effects of age on brain tissue volumes have been shown between cross-sectional and longitudinal data.[1] Another possible limitation could be the relatively low similarity index for classification of WML. This was particularly due to low similarity index for small WML. The underlying reason is that the same amount of partial-volume voxels being classified differently will have a larger effect on the similarity index of smaller WML than on the similarity index of larger WML.[24] Indeed, if we excluded two persons with smallest WML from our validation set, the similarity index for WML increased to 0.71. Furthermore, the high ICC for WML indicates that despite any potential misclassification, the relative ranking of our participants was very good.

Before interpreting our results in light of published literature, two considerations need to be addressed. Firstly, we included only non-demented persons in our study, whereas various other neuroimaging studies in elderly persons also included demented and cognitively impaired persons.[1, 25] Therefore, because of different study populations, comparison with other studies needs to be done with caution. Secondly, we used a 3D Inversion-Recovery sequence, whereas other studies usually base their classification on a T1-weighted or T2-weighted sequence.[1, 6] We did not use our T1-weighted sequence, because of inter-leaved scanning, which would complicate image processing. Differences in sequences used for tissue classification may influence comparison between studies. However, Anbeek et al. showed that inclusion of an Inversion-Recovery sequence in k-nearest-neighbor classification yielded results that were at least as good as with inclusion of the T1-weighted sequence.[19]

Studies have consistently shown smaller whole brain volumes with aging in persons above 55 years of age.[1, 13, 26] We found similar effects in our study of elderly persons. However, studies further investigating GM and WM separately have not yielded uniform results. Differences in study population, study design and - most importantly - age range under investigation might explain most discrepancies. We did not find an association between age and GM. This is in contrast to studies reporting a decline in GM volume from early adulthood onwards.[1, 4, 6, 8, 9, 12, 27] However, these studies investigated GM volume over a broad age range, whereas we focused on elderly persons specifically. Moreover, our data are supported by a recent neuroimaging study by Greenberg et al.[28] and various post-mortem studies[29-31] showing that GM decline is minimal in healthy older human and non-human primates.

We found that the volume of total WM not only decreases with age, but also in the presence of small vessel disease. DeCarli et al. reported a similar association between lacunar infarcts and whole brain volume, but did not analyze GM and total WM separately.[13] Furthermore, our data confirm results by Jernigan et al. that a larger volume of WML coincides with smaller volumes of both normal and total WM.[12] We propose two mechanisms that may influence normal WM. Firstly, normal WM transforms into WML and lacunar infarcts. Secondly, normal WM atrophies, leading to a smaller volume of total WM. The question

remains whether these are sequential processes or whether WM undergoes atrophy concomitant with lesion formation and infarction. Longitudinal studies that distinguish total WM in normal WM and WML are required to elucidate this issue.

In contrast to previous reports,[8, 9] adding a quadratic term did not improve our WM volume model. Because whole brain WM volume peaks at age 40 years, the age range of our study population (60 years and over) might explain this finding.[10, 32] Once again, this emphasizes the importance of the age range under investigation.[10]

Although we found that men had proportionally smaller BV and larger volume of total WM than women, whereas women had more GM and WML, no differences were seen between the sexes in the effect of age. This is in line with findings from two previous studies and might reflect genetic effects of sex on mean tissue volumes.[33, 34] However, data on sex differences in mean tissue volumes or in the effect of age have not always yielded consistent results.[1, 4]

Finally, we showed that current smoking, higher diastolic blood pressure and diabetes mellitus were all related to smaller brain volume. This is in line with previous studies, which reported more neurodegenerative changes in persons with more vascular damage.[35-37] We found that the effect of these determinants on brain tissue volumes was independent of each other. Previously, we found that these vascular risk factors are also related to lacunar infarcts and WML.[38, 39] Several studies reported that ischemic damage, arteriolosclerosis and hypoperfusion may underlie this relationship between vascular factors and lacunar infarcts and WML.[40] Our data suggest that similar mechanisms may also underlie brain atrophy. It is unclear whether various cardiovascular risk factors also affect GM and WM differently. We found that diabetes mellitus was more related to GM than to total WM, which is in line with two previous studies showing that diabetes mellitus is associated with cortical atrophy[41] and hippocampal atrophy,[42] but not with subcortical atrophy.[41] In contrast, current smoking and diastolic blood pressure were more related to total WM than to GM. However, these associations with GM and total WM separately need to be replicated in other population-based studies.

In conclusion, our study shows that in the elderly higher age, presence of small vessel disease, and cardiovascular risk factors are associated with a smaller whole brain volume. This is particularly due to a smaller volume of WM.

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Chapter 2.1.2

Incidental Findings on Brain MRI in the General Population

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ABSTRACT

Background: Magnetic resonance imaging (MRI) of the brain is increasingly used both in research and in clinical medicine, and scanner hardware and MRI sequences are continually being improved. These advances are likely to result in the detection of unexpected, asymptomatic brain abnormalities, such as brain tumors, aneurysms, and subclinical vascular pathologic changes. We conducted a study to determine the prevalence of such incidental brain findings in the general population.

Methods: The subjects were 2000 persons (mean age, 63.3 years; range, 45.7 to 96.7) from the population-based Rotterdam Study in whom high-resolution, structural brain MRI (1.5 T) was performed according to a standardized protocol. Two trained reviewers recorded all brain abnormalities, including asymptomatic brain infarcts. The volume of white matter lesions was quantified in milliliters with the use of automated postprocessing techniques. Two experienced neuroradiologists reviewed all incidental findings. All diagnoses were based on MRI findings, and additional histologic confirmation was not obtained.

Results: Asymptomatic brain infarcts were present in 145 persons (7.2%). Among findings other than infarcts, cerebral aneurysms (1.8%) and benign primary tumors (1.6%), mainly meningiomas, were the most frequent. The prevalence of asymptomatic brain infarcts and meningiomas increased with age, as did the volume of white matter lesions, whereas aneurysms showed no age-related increase in prevalence.

Conclusions: Incidental brain findings on MRI, including subclinical vascular pathologic changes, are common in the general population. The most frequent are brain infarcts, followed by cerebral aneurysms and benign primary tumors. Information on the natural course of these lesions is needed to inform clinical management.

INTRODUCTION

Magnetic resonance imaging (MRI) of the brain is increasingly used both in research and in clinical medicine, and scanner hardware and MRI sequences are improving. Performing MRI at higher resolution and field strength and with more sensitive sequences may lead to the detection of subtle or small brain abnormalities that would not have been detected previously. In combination with the increasing number of brain MRI scans obtained each year, these advances in MRI technology will probably result in more persons being confronted with incidental brain findings. Incidental findings are previously undetected abnormalities of potential clinical relevance that are unexpectedly discovered and unrelated to the purpose of the examination.[1] The detection of incidental findings poses various practical and ethical issues, particularly when the participants in a research study are healthy volunteers.[2] The clinical relevance and natural course of these unexpected asymptomatic findings are largely unknown and may differ markedly from those of similar symptomatic abnormalities.

Previous studies investigated incidental findings, such as brain tumors and vascular abnormalities, in healthy research volunteers or in populations of patients who underwent MRI examinations for various reasons.[3-8] Katzman et al. reported a prevalence of 1.1% for clinically serious abnormalities, such as brain tumors, in a retrospective study of a heterogeneous population of volunteers, 3 to 83 years old, who were participating in a variety of research studies.[9] To date, only one population-based study has reported the occurrence of incidental brain findings; this study showed a prevalence of 1.7%.[10, 11]

Not generally classified as incidental findings are subclinical vascular pathologic changes such as asymptomatic brain infarcts and white matter lesions, the prevalence of which is known to be high in elderly persons and to increase with age.[12-17] These lesions are potentially clinically relevant because of the increased risk of adverse neurologic events associated with them.[17-22] We report on the prevalence of incidental brain findings, including subclinical vascular pathologic changes, detected by high-resolution, state-of-the-art brain MRI in 2000 persons who participated in a population-based study.

METHODS

Source population

The subjects of this study were participants in the Rotterdam Study, a prospective, population-based cohort study initiated in 1990 among persons 55 years of age or older who were living in a suburb of Rotterdam, the Netherlands.[23] The original cohort of the Rotterdam Study (7983 participants) was expanded in 2000 and again in 2006 to include participants who were 45 years of age or older. Every 2 to 3 years, participants are invited to the research center for interviews and extensive physical examinations. Since August 2005, all participants without contraindications to MRI have been invited to undergo MRI examination as part of the Rotterdam Scan Study, a neuroimaging study embedded in the Rotterdam Study that aims to investigate the causes and consequences of age-related brain changes.

The institutional review board at Erasmus MC University Medical Center approved the study, and all participants gave written informed consent; the consent form included a paragraph on incidental findings and the option to refuse to be informed about any unexpected abnormality. All patients who had incidental findings that required follow-up evaluation or treatment had previously agreed to be informed of such findings and were referred to appropriate specialists.

Between August 1st, 2005, and February 1st, 2007, 2027 of 2227 eligible subjects (91.0%) agreed to participate in the imaging study. In 27 subjects, imaging could not be performed because of physical constraints (in 21 subjects) or technical problems (in 6 subjects). Brain imaging results were thus available for 2000 participants.

Brain MRI acquisition

All scans were obtained with a 1.5-T scanner with an eight-channel head coil (GE Healthcare). Two trained technicians performed all examinations in a standardized way. The MRI protocol was identical for all participants and included four high-resolution axial sequences: a three-dimensional, T1-weighted sequence; a two-dimensional, proton-density– weighted sequence; a two-dimensional, fluid-attenuated inversion recovery (FLAIR) sequence; and a three-dimensional, T2*-weighted gradient-recalled echo (GRE) sequence. The slice thickness was 1.6 mm for the T1-weighted, proton- density–weighted, and T2*-weighted GRE sequences (zero-padded to 0.8 mm for the T1-weighted and T2*-weighted GRE sequences) and 2.5 mm for the FLAIR sequence; all slices were contiguous. No contrast material was administered.

Assessment of incidental findings

All scans were read for incidental findings by one of two trained reviewers. The readings were usually performed within 1 day (over 90% of all scans) and at the latest 1 week after

acquisition. One reviewer was a resident in radiology, and the other a resident in neurology, with 4.5 and 2.0 years of experience in reading brain MRIs, respectively. Both reviewers were unaware of any clinical information on the subjects. The readings were performed with a digital picture archiving and communication system (PACS). Incidental findings of potential clinical relevance were defined as those requiring urgent or immediate referral, as previously described by others;[9, 10, 24] examples include brain tumors, aneurysms, subdural fluid collections, and arachnoid cysts. The diagnoses were made on the basis of MRI findings characteristic of each lesion and were not confirmed by histologic studies. Case definitions for each incidental MRI finding are detailed in the Appendix.

In addition, the presence of brain infarcts (both lacunar and cortical) was recorded. The distinction between symptomatic and asymptomatic infarcts was verified as follows. A history of stroke is obtained from each subject on entry into the Rotterdam Study.[25] Subsequently, participants are continuously monitored for incident stroke through automated linkage of the study database with files from general practitioners and hospital discharge information. All reported events are validated by an experienced neurologist.[26] White matter lesion volumes (in milliliters) were quantified with a validated automated voxel classification technique, as described elsewhere.[27] Brain findings that were not considered clinically relevant and were not recorded as incidental findings included simple sinus disease and variations from the norm, such as pineal cysts, ventricular asymmetry, and enlarged Virchow–Robin spaces.

Two experienced neuroradiologists reviewed and reached a consensus on all initially reported abnormalities. To maximize sensitivity, the threshold for reporting abnormalities on initial review was kept low. To verify the sensitivity of the initial review for detecting incidental findings, an additional 230 scans (11.5% of the total of 2000) were also read by the neuroradiologists. No brain abnormalities were detected in addition to those already recorded by the initial reviewers. This result indicates that the initial review had a very high sensitivity for detection of brain abnormalities.

The management of incidental findings was defined in a protocol that was agreed on before the start of the study. Depending on the detected abnormality and after consultation with clinicians, persons with incidental findings requiring additional clinical workup or medical treatment were referred to a relevant medical specialist (a neurosurgeon, neurologist, or internist).

Statistical analysis

We calculated the prevalence of each incidental brain finding in the study population. Multiple similar findings within one participant (e.g., more than one aneurysm or multiple asymptomatic brain infarcts) were counted as a single finding. Next, we calculated the age-specific prevalence rates of the most frequent incidental findings. For white matter lesions, we calculated the age-specific median and interquartile range.

RESULTS

The mean age of the study population was 63.3 years (range, 45.7 to 96.7), and 1049 of the subjects (52.4%) were women. Table 1 shows the prevalence of each incidental finding that was recorded. Asymptomatic brain infarcts were present in 145 persons (7.2%). Among findings other than brain infarcts, aneurysms (1.8%) were the most frequent. All aneurysms except two were located in the anterior circulation, and all except three were less than 7 mm in diameter (the smallest was 2 mm). Four aneurysms had an intracavernous location. Benign tumors were also frequent (1.6%), with meningiomas being recorded most often (0.9%). The meningiomas ranged from 5 to 60 mm in diameter, and their prevalence was 1.1% in women and 0.7% in men. Pituitary macroadenoma was present in six persons (0.3%). Vestibular schwannomas had a prevalence of 0.2%. We found one possibly malignant primary brain tumor (a low-grade glioma that was not histologically confirmed) and one case of multiple cerebral metastases in a person who in retrospect was found to have been

Table 1. Incidental findings on 2000 MRI scans*

Finding	No. (%)
Asymptomatic brain infarct†	145 (7.2)
Lacunar infarcts	112 (5.6)
Cortical infarcts	41 (2.0)
Primary tumors, benign	31 (1.6)
Meningioma	18 (0.9)
Vestibular schwannoma	4 (0.2)
Intracranial lipoma‡	2 (0.1)
Trigeminal schwannoma	1 (<0.1)
Pituitary adenoma	6 (0.3)
Primary tumors, malignant§	1 (<0.1)
Other findings	
Aneurysm	35 (1.8)
Cavernous angioma	7 (0.4)
Metastases	1 (<0.1)
Subdural hematoma	1 (<0.1)
Arachnoid cyst¶	22 (1.1)
Chiari I malformation‖	18 (0.9)
Major vessel stenosis**	9 (0.5)
Dermoid cyst of lateral orbital rim	1 (<0.1)
Fibrous dysplasia	1 (<0.1)

* The diagnoses were based on imaging only, without histologic confirmation.

† Some subjects had both lacunar and cortical infarcts.

‡ One person had quadrigeminal cistern lipoma, and one had intravestibular lipoma.

§ This finding was a possible low-grade glioma.

¶ There were 16 temporal cysts (left-to-right ratio, 3:1) and 6 infratentorial cysts.

‖ Type I Chiari malformation is defined as tonsillar herniation extending more than 5 mm below the foramen magnum.[28] The mean degree of herniation was 6.4 mm (range, 5.2 to 10.3).

** Major-vessel stenosis is defined as lack of flow void in the cavernous internal carotid artery (in seven subjects) or the vertebral artery (in two subjects).

treated for lung cancer. The finding that was medically most urgent was a large, chronic subdural hematoma in an otherwise asymptomatic person, who in retrospect was found to have had minor head trauma 4 weeks before the MRI scan. Figure 1 shows a selection of the abnormalities that were incidentally detected in this study.

None of the persons with incidental brain findings reported any symptoms, with the exception of two subjects. One person with vestibular schwannoma reported hearing loss that had been investigated 3 years earlier by computed tomography, which had not revealed any abnormalities. The other person, who had a right-sided intravestibular lipoma, had longstanding ipsilateral hearing loss that had never been evaluated.

None of the incidental findings in Table 1 were histologically or surgically confirmed, except for those in two persons for whom operative treatment was indicated. One had subdural hematoma, and the other had a 12-mm aneurysm of the medial cerebral artery.

Table 2 shows the age-specific distribution of the most frequent incidental findings. The prevalence of asymptomatic brain infarcts increased with age. The prevalence of meningiomas increased from 0.5% in 45- to 59-year-olds to 1.6% in persons 75 years of age or older. Aneurysms showed no change in prevalence with age.

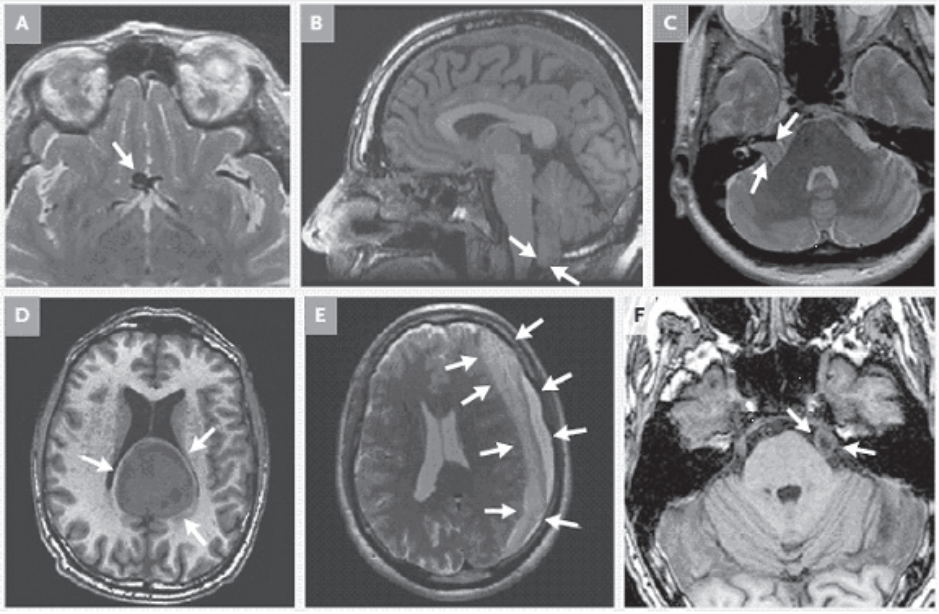

Figure 1. Incidental findings on brain MRI. Arrows indicate the abnormalities in each image. An aneurysm of the anterior communicating artery (diameter, 6 mm) is shown on the proton-density–weighted axial image in Panel A. Panel B shows a tonsillar herniation (type I Chiari malformation) more than 5 mm below the level of the foramen magnum on a T1-weighted sagittal image. A typical vestibular schwannoma with extension into the right internal auditory canal is visible on the proton-density– weighted axial image in Panel C. A large meningioma is shown on the T1-weighted axial image in Panel D. Panel E shows a large, chronic subdural hematoma on a proton-density–weighted axial image. A trigeminal schwannoma of the left fifth cranial nerve, with cystic degeneration, is shown on the T1-weighted axial image in Panel F.

Table 2. Distribution of incidental findings according to age			
Finding	**45 to 59 Yr of Age (N= 750)**	**60 to 74 Yr of Age (N = 993)**	**75 to 97 Yr of Age (N = 257)**
Asymptomatic brain infarct – no. (%)	30 (4.0)	68 (6.8)	47 (18.3)
Meningioma – no. (%)	4 (0.5)	10 (1.0)	4 (1.6)
Aneurysm – no. (%)	13 (1.7)	18 (1.8)	4 (1.6)
Volume of white matter lesions - ml			
Median	1.80	3.05	7.74
Interquartile range	1.06-3.17	1.87-5.49	2.64-16.49

The median volume of white matter lesions increased with increased age (Table 2). The distribution of white matter lesion volumes according to age category is shown in Figure 2. The proportion of persons without any white matter lesions decreased from 5.4% in 45- to 59-year-olds to 2.0% in persons 75 years of age and older. Furthermore, with increasing age, there was a greater spread in the distribution of white matter lesion volumes (Figure 2).

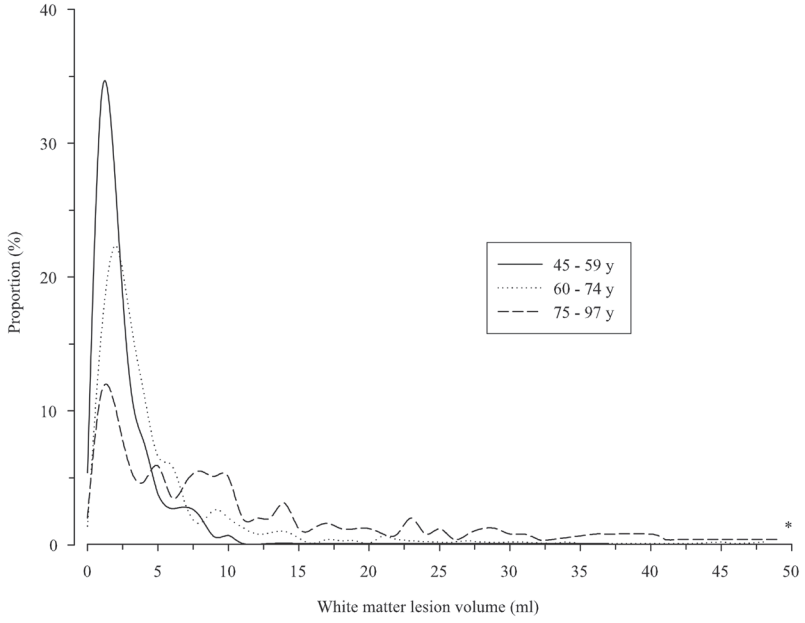

Figure 2. Age-specific distribution of white matter lesion volumes. Measured volumes of white matter lesions were rounded to the nearest milliliter before plotting. The proportion of persons with a specific volume of white matter lesions within each age category is shown on the y axis. Fourteen persons (<1%) had a white matter lesion volume of more than 50 ml. Of these persons, one was under 59 years of age, four were 60 to 74 years of age, and nine were 75 years of age or older.
*The maximum white matter lesion volume measured was 95 ml.

DISCUSSION

In the general population of persons 45 to 97 years old, we found a high prevalence of potentially clinically relevant incidental brain abnormalities, including subclinical vascular pathologic changes. The prevalence of asymptomatic brain infarcts and meningiomas increased with age, as did the volume of white matter lesions, whereas aneurysms showed no age-related increase in prevalence.

A major strength of our study is the large sample of persons 45 years of age or older. The MRI protocol was uniform for all subjects, and the reviewers were unaware of characteristics of the subjects, making detection bias unlikely. We used high-resolution, state-of-the-art imaging sequences representing the advanced imaging techniques that are increasingly used in brain research.

A potential limitation with respect to the generalizability of our study results is the fairly homogeneous composition of our geographically defined study population, which consisted mainly of white, middle-class persons.[29] Our results may not be generalizable to populations that include other ethnic or socioeconomic groups.

Another potential limitation of our study is that not all scans were read by neuroradiologists. However, all scans with abnormalities detected on initial review were reviewed again by two neuroradiologists. In addition, a randomly chosen subgroup of all scans was reviewed by two neuroradiologists, who did not detect any incidental findings missed on initial review. Therefore, our initial review by physicians who were not neuroradiologists had a very high sensitivity for the detection of brain abnormalities, and we do not think the results would have been different if the scans had been read primarily by neuroradiologists. The sensitivity may be lower when scans are read by professionals who are not medically qualified, as is reportedly the case in many research centers in the United States.[30]

The incidental brain findings in our study were all diagnosed on the basis of imaging. Pathological confirmation of presumed brain tumors was not obtained, since none of these tumors required surgery after referral of the subject. However, the imaging characteristics of all lesions listed in Table 1 were typical and are usually considered diagnostic (see the Supplementary Appendix).

We did not use contrast-enhanced MRI. Because our study population consisted of volunteers without neurologic symptoms who were participating in a research study, the risks associated with the administration of contrast material were not considered warranted. However, the effect of the absence of contrast material, if any, would have been to leave some small lesions undetected, which would have resulted in an underestimate of the prevalence of incidental findings.

The prevalence of subclinical vascular pathologic changes in our population was high and increased with advancing age. This finding was not unexpected, since age-related changes, such as asymptomatic brain infarcts and white matter lesions, have been reported

to be very frequent in the general elderly population.[12, 13, 15-17, 31] Although such changes have been shown to be associated with increased risks of stroke and cognitive decline,[18, 20, 32] preventive therapies for patients with these MRI findings have not been evaluated in randomized trials.

The prevalence of incidental brain findings other than subclinical vascular pathologic changes in our population was much higher than that reported in previous studies,[8-10, 24] even when the subjects were of similar age to the patients in our study.[10] We found an especially high prevalence of small aneurysms.[4, 9, 10, 24] This difference can partly be explained by differences among study populations, since aneurysms are very infrequent in children and young adults. However, the population- based study by Yue et al. showed aneurysms in only 0.11% of persons 65 years of age or older.[10] We feel that a more likely explanation for the difference is that our scanning protocol, especially the high-resolution, proton-density–weighted sequence (Figure 1A), permitted very good visualization of the circle of Willis as compared with conventional T1-weighted and T2-weighted sequences. Of course, the use of even more sensitive sequences, such as magnetic resonance angiography, might have resulted in the detection of even smaller aneurysms. However, in a systematic review of autopsy and angiographic studies, Rinkel et al. concluded that aneurysms can be found in approximately 2% of adults without risk factors for subarachnoid hemorrhage,[33] a proportion very close to the 1.8% detected by MRI in our study.

Meningiomas and small aneurysms were highly prevalent in our study population of persons 45 years of age or older. The rate of growth of meningiomas is typically slow,[34, 35] and most meningiomas remain asymptomatic throughout life, which explains why 50% of all meningiomas are discovered at autopsy.[36] The prevalence of meningiomas found at autopsy in persons over 60 years of age is 3%, and the majority of the lesions are less than 1 cm in diameter.[37] Nevertheless, it is generally believed that asymptomatic meningiomas require close clinical and radiologic followup to rule out rapidly enlarging tumors.[34, 38] The current practice of many clinicians is to perform MRI yearly for at least 2 to 3 years to ascertain that rapid tumor growth does not occur. If this were done for all persons incidentally found to have meningiomas, many MRI examinations would be performed in otherwise healthy asymptomatic persons. In view of the resulting medical costs, as well as the psychological burden for those undergoing examination, it would be of great interest to review these guidelines on the basis of the natural course of meningiomas incidentally found on brain MRI.

Guidelines for the management of small aneurysms might also be reviewed. More than 90% of unruptured, asymptomatic aneurysms found by means of autopsy or angiography are less than 10 mm in diameter.[33,39] In our study, all but three aneurysms were smaller than 7 mm, and all but two were located in the anterior circulation. The reported risk of rupture for aneurysms of this size in the anterior circulation over a period of 4 years is 0%.[40] This finding was based on follow- up of a group of patients who had no history of subarachnoid

hemorrhage. However, in this group there was an overrepresentation of persons with a family history of aneurysm and of persons with symptoms that had led to the detection of the unruptured aneurysm.[40] The risk of rupture associated with asymptomatic aneurysms in the general population would be expected to be even lower than the reported risk in the described patient population.[33] Preventive surgery or treatment of risk factors may thus not be indicated in the general population, and the benefit of longer follow-up has not yet been proven.[41] Therefore, persons in our study with aneurysms of the anterior circulation that were under 7 mm in diameter were not referred for follow-up or medical treatment.

Several large, population-based MRI studies in the elderly are ongoing,[11, 16, 42-45] and more will be conducted because of the increasing scientific interest in age-related brain diseases such as dementia. Moreover, imaging at higher MRI field strengths and with increased resolution, as well as the use of new MRI sequences that are more sensitive to subtle structural changes, will probably increase the number of small brain abnormalities detected. Incidental findings from brain MRI in middle-aged and elderly persons will therefore become an important issue that should be considered in designing studies.The present study, as well as some previous studies,[9,10] provides information on the prevalence of clinically asymptomatic brain abnormalities.This information is especially important in view of the ethical and practical issues involved in the management of incidental findings.[1, 2]

In conclusion, incidental findings on brain MRI in the general population are common. The most frequent findings are brain infarcts, followed by cerebral aneurysms and benign primary tumors. Such findings should be anticipated in the design of research protocols and the use of neuroimaging in clinical practice. Information on the natural course and prognosis of these lesions is needed to inform clinical management.

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APPENDIX TO CHAPTER 2.1.2

Imaging diagnosis	Case definition based on MRI characteristics
Asymptomatic brain infarct	
Lacunar infarct	Focal parenchymal lesion ≥ 3 mm and < 15 mm in size, with the same signal characteristics as cerebrospinal fluid on all sequences, and -when located supratentorially- with a hyperintense rim on the FLAIR images.1-3 No involvement of cortical grey matter. Commonly located in the basal ganglia, internal capsule, pons and corona radiata. Differentiation from Virchow-Robin (VR) spaces is based on signal intensity (absence of hyperintense rim on FLAIR images), shape (VR-spaces are more linear or lobulated in shape) and location (VR-spaces are often located around anterior commissure or near vertex of the brain).4, 5
Subcortical infarct	Same MRI characteristics as lacunar infarct, but ≥ 15 mm in size.
Cortical infarct	Focal parenchymal lesion with involvement of cortical grey matter, with the same signal characteristics as cerebrospinal fluid on all sequences, and -when located supratentorially- with a hyperintense rim on the FLAIR images.1-3 Tissue loss of variable magnitude present, visible as prominent adjacent sulci and ipsilateral ventricular enlargement.6
Primary tumors, benign	
Meningioma	Extra-axial lesion. Iso- or hypointense to grey matter on T1-weighted images, variable signal intensity on PD-weighted images. Calcifications (hypointense on T1-weighted and PD-weighted images) within lesion and/or hyperostosis of underlying bone may be present. Usually broad dural basis. When large, these lesions may cause moderate vasogenic edema in underlying brain tissue).6
Vestibular schwannoma	Extra-axial lesion. Iso- or hypointense to grey matter on T1-weighted images. Located in the internal auditory canal, with variable extension into the cerebellopontine angle. Often with widening of the internal auditory canal when large. Typical "ice cream cone" appearance.6 Can show cystic changes (visible as high signal intensity on PD-weighted images).7
Intracranial lipoma	Lesion with the same signal characteristics as subcutaneous fat on all sequences. Sometimes with intralesional vessels seen as flow voids.8, 9
Trigeminal schwannoma	Extra-axial lesion with signal characteristics similar to vestibular schwannoma except the course follows that of the 5th cranial nerve.6, 10
Pituitary macroadenoma	Intrasellar mass, extending suprasellar and/or parasellar, frequently causing deviation of the pituitary stalk. May extend upward toward the optic chiasm. The normal pituitary gland may not be identified. Signal intensity usually isointense to grey matter on all sequences, but the lesion may show cystic changes (cystic macroadenoma; high signal intensity on PD-weighted images).6
Primary tumors, malignant	
Low-grade glioma	Diffuse lesion with mass effect and signal changes: hypointense relative to surrounding brain on T1-weighted images, hyperintense on PD-weighted and FLAIR images. No signs of necrosis or hemorrhage.6
Other findings	
Aneurysm	The presence of aneurysms is evaluated on PD-weighted images, on which arterial structures are visualized as flow voids (black). Aneurysms are defined as blind-ending, well delineated focal arterial out-pouchings with a saccular shape. Location usually in cavernous internal carotid artery or circle of Willis. Commonly located at vessel bifurcations.6

Cavernous angioma	"Popcorn-like", smoothly circumscribed, well-delineated parenchymal lesion. Complex reticulated core of mixed signal intensities, representing hemorrhage in various stages of evolution. Low-signal-intensity hemosiderin rim completely surrounding the lesion on both T1-weighted and PD-weighted images. On T2* GRE imaging, paramagnetic properties of hemosiderin cause a focus of signal loss. No feeding artery or draining vein demonstrated.6
Metastases	Multifocal parenchymal round lesions with mass effect. Generally iso- to mildly hypointense on T1-weighted images, hyperintense on PD-weighted images. Variable amount of edema surrounding each lesion. Hemorrhage may be present in some lesions (causing susceptibility artifacts on T2* GRE images).6
Chronic subdural hematoma	Crescent-shaped extra-axial fluid collection. Hyperintense signal intensity on PD-weighted images. Often not homogeneous in signal intensity due to presence of blood in different stages, with septae separating different blood products. The extra-axial fluid collection does not cross dural attachments, but does cross sutures.6
Arachnoid cyst	Sharply-demarcated well-defined extra-axial cystic lesion exhibiting isointense signal to cerebrospinal fluid on all sequences (including on FLAIR images). No internal architecture.Typical locations are temporal or infratentorial (cerebellopontine angle, cisterna magna).6
Chiari I malformation	Tonsillar herniation extending more than 5 mm below the foramen magnum. The plane of the foramen magnum is defined on sagittal T1-weighted images by a line connecting the basion and opisthion, and degree of tonsillar herniation is measured perpendicular from this line to the most inferior aspect of the cerebellar tonsils visible on all sections.11
Major vessel stenosis	Absence of flow void on T1-weighted and PD-weighted images in carotid or vertebral artery.12
Extra-cranial dermoid cyst	Extra-cranial lesion usually located around bony sutures (typical near superolateral orbital rim).Well-defined lesion, usually hypointense on T1-weighted and hyperintense on PD-weighted images. May exhibit a fat-fluid level. Shows bony remodelling without destruction.13
Fibrous dysplasia	Bony expansion with intact but thickened cortex. Low signal intensity on both T1-weighted and PD-weighted images, as well as on FLAIR images.6, 14 No extension into soft tissue.Typical locations in the skull are frontal, sphenoid, maxillary, and ethmoidal bones.14

Abbreviations: FLAIR fluid-attenuated inversion recovery; PD proton density; GRE gradient-recalled echo.

Note that the PD-weighted sequence used in our MR protocol is a fast spin echo sequence with a long repetition time (12,300 ms), which results in hyperintense signal of (cerebrospinal) fluid, comparable to the tissue-fluid contrast seen in T2-weighted sequences.

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Chapter 2.2

Kidney Function is related to Cerebral Small Vessel Disease

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ABSTRACT

Background and purpose: Poor kidney function, as measured by glomerular filtration rate (GFR), is closely associated with presence of glomerular small vessel disease. Given the hemodynamic similarities between the vascular beds of the kidney and the brain, we hypothesized an association between kidney function and markers of cerebral small vessel disease on magnetic resonance imaging (MRI). We investigated this association in a population-based study of elderly persons.

Methods: We measured GFR using the Cockcroft-Gault equation in 484 participants (60-90 years) from the Rotterdam Scan Study. Using automated MRI-analysis we measured global as well as lobar and deep volumes of grey matter and white matter, and volume of WML. Lacunar infarcts were rated visually. Volumes of deep white matter and WML, and presence of lacunar infarcts reflected cerebral small vessel disease. We used linear and logistic regression models to investigate the association between GFR and brain imaging parameters. Analyses were adjusted for age, sex and additionally for cardiovascular risk factors.

Results: Persons with lower GFR had less deep white matter volume (difference in standardized volume per SD decrease in GFR: -0.15 (95% CI -0.26 to -0.04)), more WML (difference per SD decrease in GFR: 0.14 (95% CI 0.03 to 0.25)), and more often lacunar infarcts, although the latter was not significant. GFR was not associated with grey matter volume or lobar white matter volume. Additional adjustment for cardiovascular risk factors yielded similar results.

Conclusion: Impaired kidney function is associated with markers of cerebral small vessel disease as assessed on MRI.

INTRODUCTION

Poor kidney function is highly prevalent in the general elderly population.[1, 2] It often remains subclinical and is then only identified by measuring a decreased glomerular filtration rate (GFR).[3] Poor kidney function is associated with features of large vessel disease, such as hypertension, arterial stiffness, and ischemic heart disease.[4-6] Moreover, kidney dysfunction is also characterized by glomerular endothelium dysfunction and lipohyalinosis, both of which are features of small vessel disease in the kidney.[7]

In the elderly, small vessel disease is also abundantly present in the brain.[8, 9] White matter lesions (WML), lacunar infarcts, and subcortical atrophy are markers of cerebral small vessel disease that are visible on magnetic resonance imaging (MRI)[10] and that increase the risk of stroke, cognitive decline and dementia.[11-13] Given the hemodynamic similarities between the vascular beds of the kidney and the brain,[14] small vessel disease in the kidney may be indicative of presence of small vessel disease in the brain. However, data on the relationship between kidney function and MRI-markers of cerebral small vessel disease are scarce. Two studies showed that decreased kidney function was associated with an increased prevalence of subclinical brain infarcts on MRI,[15, 16] which are mostly lacunar infarcts.[9] However, they did not investigate WML or subcortical atrophy. Recently, the Northern Manhattan Study presented data that showed an association between kidney function and WML.[17]

We hypothesized an association between kidney function, as measured by GFR, and MRI-markers of cerebral small vessel disease and investigated this association in the population-based Rotterdam Scan Study.

MATERIALS AND METHODS

Study population

The Rotterdam Study is a large population-base cohort study in the Netherlands that started in 1990 and investigates the prevalence, incidence and determinants of chronic diseases in the elderly.[18] In 1995 to 1996 we randomly selected 965 living members (60-90 years of age) of the cohort in strata of sex and age (5 years) to participate in the Rotterdam Scan Study, designed to investigate age-related brain abnormalities on MRI.[19] After excluding persons who were demented or had MRI contraindications, 832 persons were eligible and invited. Among these, 563 persons gave their written informed consent and participated in the study, which included physical examination, blood sampling and an MRI scan of the brain (response 68%). Participants were in general healthier than non-participants.[20] Of the 563 participants, 52 developed claustrophobia during MRI acquisition. Twenty-one datasets were unusable due to excessive ghosting artifacts (n=5), scanning outside the range of coil sensitivity (n=10), or other reasons (n=6), leaving a total of 490 participants with complete and usable MRI data.[21] The study protocol was approved by the medical ethics committee of the Erasmus MC, Rotterdam, the Netherlands. The large majority of participants (>97%) were of Caucasian ethnicity.

Measurement of glomerular filtration rate

Non-fasting blood was collected and centrifuged within 30 minutes at 3000 rotations per minute for 10 minutes. Subsequently the serum was stored at -20°C for 1 week, until serum creatinine level was assessed by a nonkinetic alkaline picrate (Jaffe) method[3] (Kone Autoanalyzer, Kone Corporation, Espoo, Finland and Elan, Merck, Darmstadt, Germany). The method was standardized against high performance liquid chromatography. The within-run precision was >98.5% and the day-by-day precision was >95.0%. Creatinine clearance was computed with the Cockcroft-Gault equation,[22] corrected with a factor 0.9, and standardized for 1.73 m^2 body surface area using the Dubois[23] formula: GFR = (140-age{years}) (weight{kg} x 1.23) (0.85 if female) (serum creatinine {µmol/l})$^{-1}$ (0.9) (1.73) (weight{kg})$^{-0.425}$ (height{cm})$^{-0.725}$ (0.007184)$^{-1}$. Creatinine clearance generally exceeds GFR by 10-15% because of additional urinary creatinine excretion due to tubular secretion.[24] The Cockroft Gault estimate of GFR was therefore additionally corrected with a factor of 0.9. Serum creatinine could not be assessed in 6 of the 490 persons due to technical difficulties, leaving 484 persons in our analysis.

MRI acquisition

MRI scans of the brain were performed on a 1.5-Tesla MRI System (VISION MR, Siemens AG, Erlangen, Germany). The protocol included T1-weighted, proton-density weighted and T2-weighted scans.[20] Furthermore, a high-resolution, inversion-recovery double contrast,

3-D HASTE sequence was acquired.[21] We used the proton-density,T2-weighted and the first HASTE module (HASTE-Odd) for our multi-spectral volumetry.

Multi-spectral brain tissue volumetry

Data were stored onto a Linux Workstation. Preprocessing steps and the classification algorithm have been described.[21, 25] In summary, preprocessing included co-registration, non-uniformity correction and variance scaling.Afterwards, we used the k-nearest-neighbor (kNN) classifier to classify voxels into cerebrospinal fluid (CSF), grey matter (GM), normal white matter (WM), and WML.[26] In order to minimize any misclassification of partial volume voxels as WML around cortical GM, we registered a manually created mask, within which voxels could be classified as WML. Using the kNN-classifier infarcts are classified as CSF and are not included in the volume of WML.

Using non-linear transformation, non-cerebral tissues (e.g. eyes, skull, dura) were stripped.[27, 28] Volumes were calculated by summing all voxels of a single tissue class and multiplying by the voxel volume.

Validation methods and results have been described and showed very good to excellent agreement between automated classification and manual classification used as reference.[21, 25]

For differentiation between lobar and deep brain tissue volumes, we first created a template scan, in which the lobar and deep regions were labeled according to a slightly modified version of the segmentation protocol as described by Bokde et al.[29, 30] Figure 1 shows an example of this segmentation, which uses anatomical landmarks and cerebral fissures as boundaries and distinguishes the lobar regions from a deep central region (i.e. the area around the ventricles, which comprises the basal ganglia, insular cortex, corpus

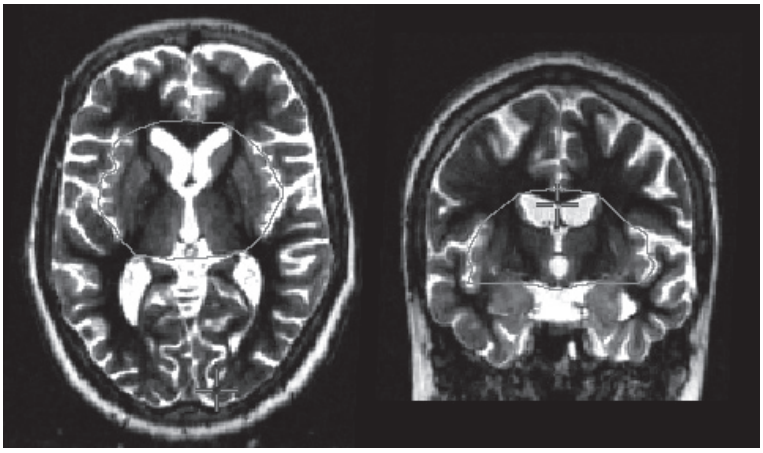

Figure 1. HASTE-Odd sequence, in which the boundary (red line) between the deep and lobar brain regions is delineated, according to the protocol by Bokde et al.[29, 30] See this figure in color in the Appendix.

callosum and the white matter in this region). The volume of the deep region reflects subcortical atrophy. Subsequently, we used validated non-rigid transformation to transform this template to each brain.[27, 28]

Rating of lacunar infarcts

Lacunar infarcts were rated visually as focal hyperintensities on T2-weighted images, 3 mm in size or larger and with a corresponding prominent hypointensity on T1-weighted images. We used the linear aspect of dilated perivascular spaces and their characteristic location around the anterior commissure to distinguish these from lacunar infarcts. Intrarater agreement for detection of infarcts was good (κ=0.80).[31]

Cardiovascular determinants

Blood pressure was measured twice in sitting position on the right arm with a random-zero sphygmomanometer. We used the average of these 2 measurements in the analyses. Diabetes mellitus was defined as a random or post-load glucose level of 11.1 mmol/l or higher, or use of oral blood glucose lowering drugs or insulin. Total cholesterol, high-density lipoprotein cholesterol, and C-reactive protein were measured in non-fasting serum with an automated enzymatic procedure. Plasma homocysteine was determined by fluorescence polarization immunoassay in an IMx analyzer (Abbott Laboratories, Chicago, IL). History of myocardial infarction was positive if a participant had reported a myocardial infarction that was confirmed by ECG or medical records. Use of blood pressure-lowering medication and smoking history were assessed during a home interview. The number of pack years of smoking was calculated by multiplying the number of cigarette packs smoked per day by the number of years smoked.

Statistical analysis

All volumes were expressed as percentage of intra-cranial volume (= CSF + GM + normal WM + WML) to correct for individual head-size differences. Whole brain volume was defined as intra-cranial volume minus CSF volume. Total WM was defined as the sum of normal WM and WML. WML were natural log transformed because of skewness of the untransformed measure.

Apart from global brain tissue volumes, we also assessed lobar and deep brain tissue volumes. To enable better comparison between the effects of kidney function on different tissue types we calculated z-scores for each participant for each tissue type separately (z-score = individual tissue volume minus mean tissue volume divided by the standard deviation).

With multiple linear regression we first investigated the association of quartiles of GFR with brain tissue volumes and WML volume. Persons in the highest quartile of GFR (indicating best kidney function) were taken as reference category. We then investigated the association of GFR continuously per standard deviation (SD) decrease with brain tissue

volumes and WML volume. We first examined global brain tissue volumes and subsequently lobar and deep tissue volumes separately. With logistic regression we investigated the association of GFR with lacunar infarcts.

All analyses were adjusted for age and sex and additionally for systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, diabetes mellitus, pack years of smoking, previous myocardial infarction, homocysteine, total cholesterol, high-density lipoprotein cholesterol and C-reactive protein.

Finally, we repeated the analyses after adjusting for or excluding persons with a cortical infarct on MRI (n=25).

RESULTS

Table 1 shows the characteristics of the study population. Figure 2 shows the associations between quartiles of kidney function and z-scores of global brain tissue volumes. Table 2 shows these associations using GFR continuously per SD decrease. Persons with low GFR had a smaller brain volume (difference in brain volume, expressed as percentage of intra-cranial volume, per standard deviation (SD) decrease in GFR: -0.35% (95% confidence interval (CI) -0.68% to -0.03%)). This smaller brain volume was not due to smaller GM volume, but rather due to smaller total and normal WM volume (figure 2 and table 2). Also, persons with low GFR had a larger volume of WML (difference in WML volume, expressed as percentage of intra-cranial volume, between lowest and upper quartile of GFR 0.47% (95% CI 0.02% to 0.92%); difference per SD decrease 0.19% (95% CI 0.03% to 0.36%)) (see also figure 2 and table 2).

Table 1. Characteristics of the study population.

	Total cohort, n=484
Age, year	73.4 (7.8)
Women, n	245 (51%)
Systolic blood pressure, mmHg	145.7 (20.5)
Diastolic blood pressure, mmHg	76.5 (11.5)
Blood pressure-lowering medication use, n	185 (38%)
Diabetes mellitus, n	24 (5%)
Smoking, pack years	20.4 (24.7)
History of myocardial infarction, n	37 (8%)
C-reactive protein, mg/l	3.71 (6.22)
Homocysteine, µmol/l	11.96 (4.47)
Total cholesterol, mmol/l	5.88 (1.05)
High-density lipoprotein cholesterol, mmol/l	1.28 (0.36)
Serum creatinine, µmol/l	89.9 (20.6)
Glomerular filtration rate, ml/min/1.73m²	54.8 (13.0)

Values are means (standard deviation) or numbers (percentages).

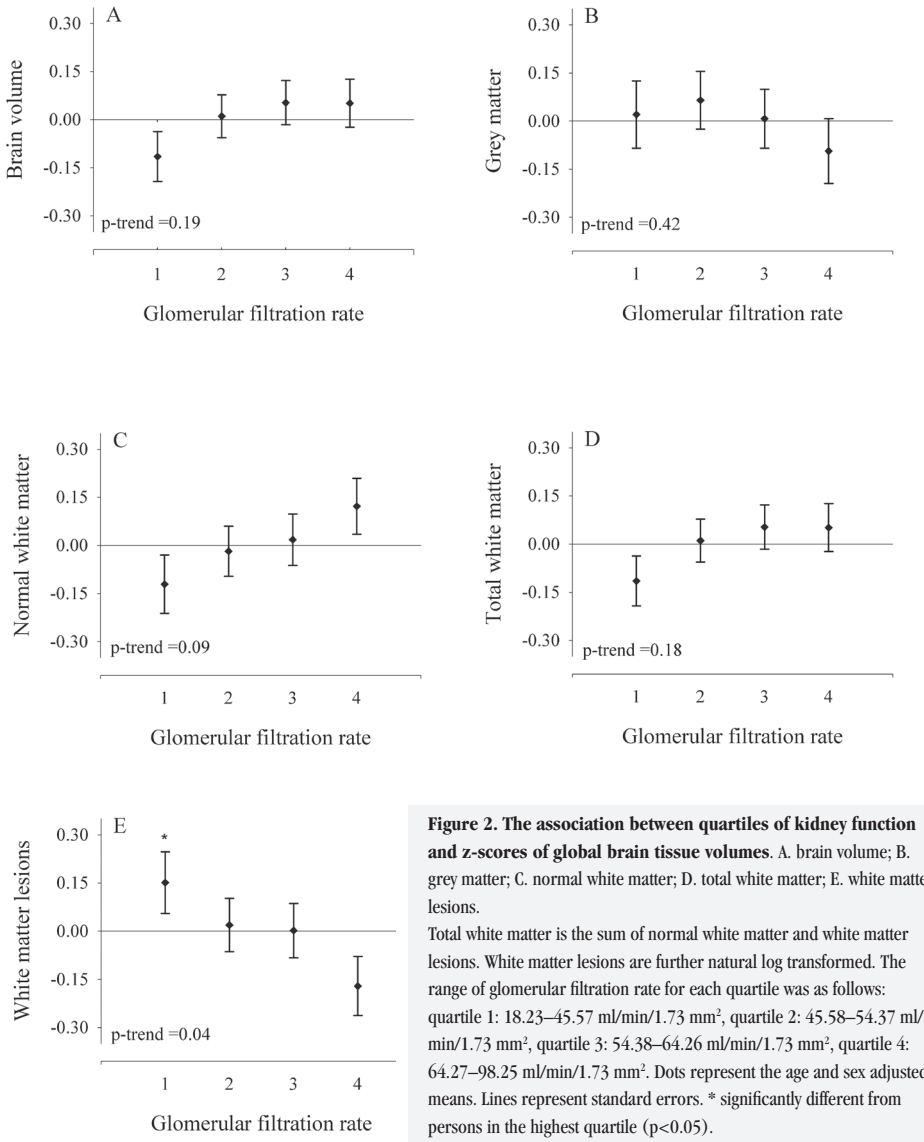

Figure 2. The association between quartiles of kidney function and z-scores of global brain tissue volumes. A. brain volume; B. grey matter; C. normal white matter; D. total white matter; E. white matter lesions.
Total white matter is the sum of normal white matter and white matter lesions. White matter lesions are further natural log transformed. The range of glomerular filtration rate for each quartile was as follows: quartile 1: 18.23–45.57 ml/min/1.73 mm^2, quartile 2: 45.58–54.37 ml/min/1.73 mm^2, quartile 3: 54.38–64.26 ml/min/1.73 mm^2, quartile 4: 64.27–98.25 ml/min/1.73 mm^2. Dots represent the age and sex adjusted means. Lines represent standard errors. * significantly different from persons in the highest quartile ($p<0.05$).

Investigating lobar and deep tissue volumes separately showed that decreased GFR was associated with both smaller lobar and deep WM volume. However, this association was weak for lobar WM, whereas it was very strong for deep WM (table 3 and figure 3). GFR was also related to lobar WML, and to a somewhat lesser extent deep WML (table 3). We did not find any association between GFR and either lobar or deep GM volume.

When additionally adjusting for cardiovascular risk factors, the associations attenuated marginally, but GFR was still related to volume of WML, to deep WM volume, and (borderline) to brain volume (tables 2 and 3).

Table 2. Relationship between kidney function and z-scores of brain tissue volumes.

Glomerular filtration rate	Brain volume	Grey matter	Normal white matter	Total white matter	White matter lesions
Per SD decrease, Model I	-0.10 (-0.18;-0.01)	0.02 (-0.10;0.14)	-0.10 (-0.20;0.01)	-0.08 (-0.18;0.03)	0.14 (0.03;0.25)
Per SD decrease, Model II	-0.09 (-0.19;0.01)	0.02 (-0.12;0.16)	-0.08 (-0.20;0.04)	-0.04 (-0.17;0.08)	0.16 (0.04;0.29)

Values represent difference in z-scores of brain tissue volumes per standard deviation decrease in kidney function. Total white matter is the sum of normal white matter and white matter lesions. White matter lesions are further natural log transformed. SD standard deviation.

Model I: adjusted for age, sex.

Model II: adjusted for age, sex, systolic and diastolic blood pressure, blood pressure-lowering medication use, diabetes mellitus, smoking, C-reactive protein, homocysteine, total cholesterol, high-density lipoprotein cholesterol, and previous myocardial infarction.

Table 3. Relationship between kidney function and z-scores of lobar and deep white matter volume.

Glomerular filtration rate	Normal white matter, lobes	Total white matter, lobes	White matter lesions, lobes	Normal white matter, deep	Total white matter, deep	White matter lesions, deep
Per SD decrease, Model I	-0.08 (-0.19;0.02)	-0.06 (-0.17;0.04)	0.15 (0.04;0.26)	-0.17 (-0.28;-0.07)	-0.15 (-0.26;-0.04)	0.10 (-0.01;0.21)
Per SD decrease, Model II	-0.07 (-0.19;0.05)	-0.03 (-0.16;0.09)	0.18 (0.05;0.30)	-0.17 (-0.29;-0.04)	-0.13 (-0.26;0.00)	0.11 (-0.01;0.24)

Values represent difference in z-scores of brain tissue volumes per standard deviation decrease in kidney function. Total white matter is the sum of normal white matter and white matter lesions. White matter lesions are further natural log transformed. SD standard deviation.

Model I: adjusted for age, sex.

Model II: adjusted for age, sex, systolic and diastolic blood pressure, blood pressure-lowering medication use, diabetes mellitus, smoking, C-reactive protein, homocysteine, total cholesterol, high-density lipoprotein cholesterol, and previous myocardial infarction.

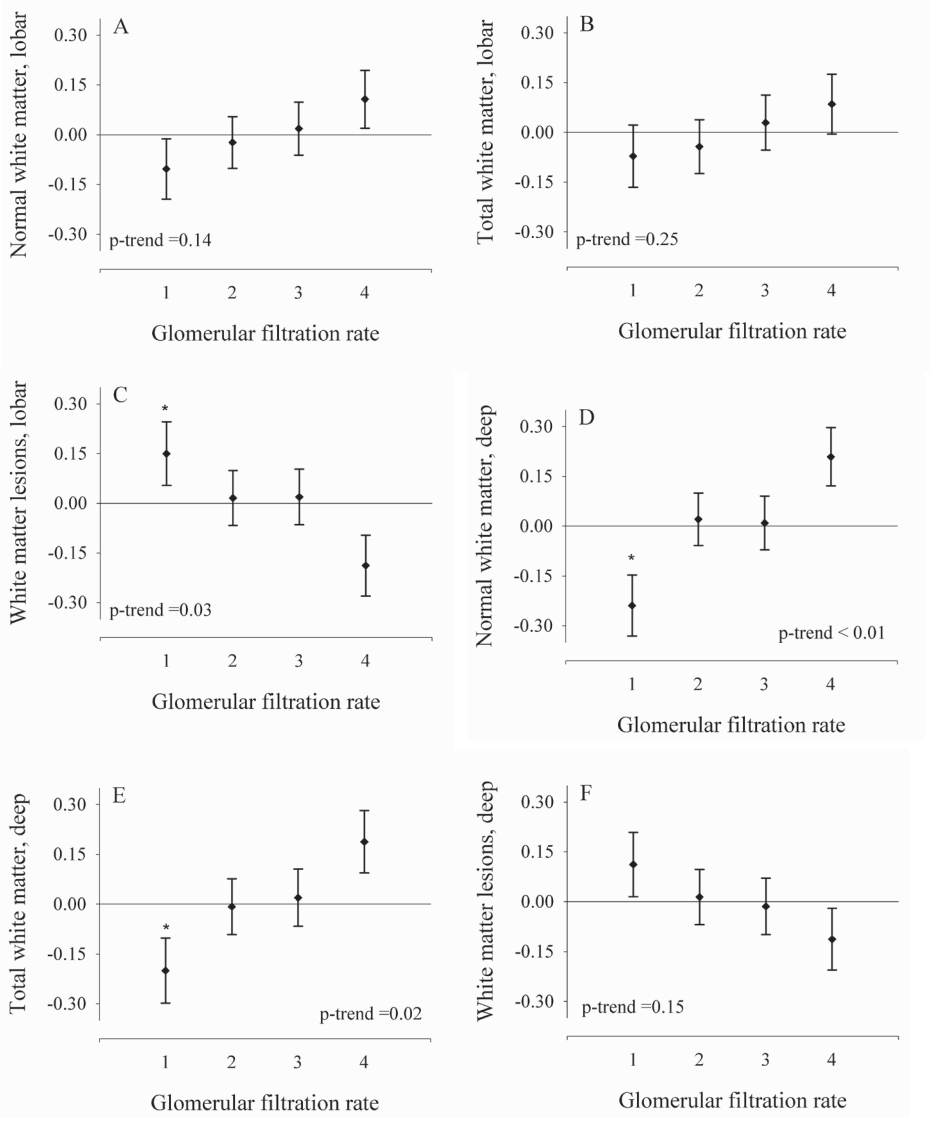

Figure 3. The association between quartiles of kidney function and z-scores of lobar and deep white matter volumes.
A. normal white matter, lobar; B. total white matter, lobar; C. white matter lesions, lobar; D. normal white matter, deep; E. total white matter, deep; F. white matter lesions, deep.
Total white matter is the sum of normal white matter and white matter lesions. White matter lesions are further natural log transformed. The range of glomerular filtration rate for each quartile was as follows: quartile 1: 18.23–45.57 ml/min/1.73 mm^2, quartile 2: 45.58–54.37 ml/min/1.73 mm^2, quartile 3: 54.38–64.26 ml/min/1.73 mm^2, quartile 4: 64.27–98.25 ml/min/1.73 mm^2. Dots represent the age and sex adjusted means. Lines represent standard errors. * significantly different from persons in the highest quartile ($p<0.05$).

Finally, persons with lower GFR had a higher prevalence of lacunar infarcts, although this was not statistically significant (age and sex adjusted prevalence odds-ratio of lacunar infarcts per SD decrease in GFR: 1.11 (95% CI 0.81 to 1.51)).

Repeating the analyses after adjusting for or excluding persons with a cortical infarct on MRI did not change any of the associations. Finally, separate analyses for men and women yielded no consistent results different from the overall analyses.

DISCUSSION

In this population-based study we found that persons with a decreased kidney function, as measured by low GFR, had smaller brain volume, smaller deep WM volume and more WML. GFR was not associated with GM volume or lobar WM volume. These associations were independent of cardiovascular risk factors.

Strengths of our study include the population-based setting, the large sample size of elderly persons aged 60 years and older, and our focus on various subclinical manifestations of cerebral small vessel disease. Moreover, the automated MR-analysis not only allowed us to accurately quantify GM and WM atrophy, but also to investigate lobar and subcortical brain atrophy separately.

Before interpreting our data some methodological issues need to be considered. The study is based on a cross-sectional study design, which limits the interpretation of our results with respect to cause and effect. Another consideration is that we used the Cockcroft Gault equation to estimate GFR and not the abbreviated Modification of Diet in Renal Disease (MDRD).[32] However, the MDRD equation has been developed in a population for which a large part of our participants would not meet inclusion criteria.[33] Therefore, using the MDRD in our population would yield misclassified measures of kidney function and would lead to dilution of the associations. For this reason and because participants were predominantly of only one ethnicity, we chose to use the Cockcroft-Gault equation in our present study. We measured serum creatinine only once, ignoring possible intra-individual fluctuations in serum creatinine levels. This may have caused our estimates to be slightly underestimated. Furthermore, serum creatinine is influenced by nonrenal factors and additional measurement of urinary albumin might have improved the sensitivity and specificity of our assessment of kidney function. Also, cystatin C is considered a superior measure of kidney function to serum creatinine. However, neither urinary albumin nor cystatin C were measured in our study.

We cannot exclude that in some cases lacunar infarcts may have been misclassified as dilated perivascular spaces and vice versa. However, given that no association has been reported yet between kidney function and dilated perivascular spaces we feel that any misclassification would probably be random and would lead to an underestimation of the true effect.

We defined subcortical (deep) brain regions according to the protocol by Bokde et al,[29, 30] which could be criticized for using arbitrarily defined borders between lobar and deep regions. Insular cortex for example is included in the deep brain region, whereas white

matter adjacent to occipital horns for example is not. We are aware that this division may not fully correspond to the 'true' position of the borders, and might not completely disentangle the separate effects of kidney function on lobar and deep brain regions. However, because the 'true' position of the borders itself is still largely unknown, we chose to apply a protocol that was designed for its practical use in population-based studies.

Several studies have shown that poor kidney function is associated with cardiovascular complications due to large-vessel disease, such as arterial calcification, heart failure, myocardial infarction and cardiac mortality.[4, 14, 34, 35] Only a few studies investigated kidney function specifically in relation to cerebrovascular disease and found that poor kidney function indicated an increased risk of clinical and subclinical stroke.[15, 16, 36, 37]

We found that decreased GFR was related to WML, subcortical atrophy, and to a lesser extent lacunar infarcts. We hypothesize that small vessel disease may underlie this association. The vascular beds of both the kidney and the brain have very low resistance and are passively perfused at high flow throughout systole and diastole.[14] Because of these unique features, which are not present in other organs, the blood vessels in the kidney and brain are highly susceptible to fluctuations in blood pressure and flow. Indeed, high blood pressure and other vascular risk factors have been shown to lead to glomerular lipohyalinosis and endothelium dysfunction, both of which are characteristics of small vessel disease in the kidney.[7, 38] Lipohyalinosis and endothelium dysfunction are also underlying features of WML and lacunar infarcts in the brain.[39] Moreover, because cerebral small vessel disease affects deep perforating arterioles, it is also characterized by atrophy in this deep subcortical region.[40] This is reflected in our dataset by the relationship between GFR and deep WM atrophy.

We did not find any association between GFR and GM volume. This observation is in line with our previous report showing that cardiovascular risk factors, such as diastolic blood pressure and smoking, were more related to WM atrophy than to GM atrophy.[21]

Previously, we have reported that several cardiovascular risk factors are associated with cerebral small vessel disease, including blood pressure,[20] CRP,[41] and homocysteine.[42] We found that adjustment for such cardiovascular risk factors only marginally changed the associations of GFR with cerebral small vessel disease. This could mean that these associations are not mediated by these risk factors, but by a different mechanism. A possibility is that GFR reflects risk factors for cerebral small vessel disease that we did not measure in our study, e.g. genetic factors. Another explanation could be that GFR is a better marker of small vessel disease than these concomitantly measured cardiovascular risk factors. However, more studies are needed to elucidate the exact mechanism underlying the association of GFR with cerebral small vessel disease.

In conclusion, our study shows that impaired kidney function, as measured by decreased GFR, is related to subclinical markers of cerebral small vessel disease, independent of cardiovascular risk factors. Therefore, GFR might be used as an easily measurable indicator of

cerebral small vessel disease. Moreover, given that cerebral small vessel disease is related to an increased risk of stroke, cognitive decline and dementia,[11-13] our data provide important information in addition to the known risk of adverse cardiac outcomes in persons with poor kidney function. Thus, our study further emphasizes the importance of identifying those with subclinical kidney disease. These persons might then benefit from installment of proper therapy. However, more studies are needed to investigate the extent to which any intervention can be beneficial.

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Chapter 2.3

Unrecognized Myocardical Infarction and Structural Brain Changes

Chapter 2.3.1

Unrecognized Myocardial Infarction and the Risk of Stroke. The Rotterdam Study

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ABSTRACT

Objective: To investigate the relationship between unrecognized myocardial infarction and the risk of stroke in a population-based cohort study.

Methods: We followed 6,439 participants from the Rotterdam Study for stroke until January 2002. Participants were free from stroke and presence of myocardial infarction was assessed at baseline (1990-1993). We calculated hazard ratios of stroke for persons with unrecognized or recognized myocardial infarction compared to persons without myocardial infarction. Analyses were adjusted for age, sex and cardiovascular risk factors.

Results: In 52,915 person-years of follow-up, 505 strokes occurred. Recognized myocardial infarction was only borderline associated with an increased risk of stroke. Unrecognized myocardial infarction increased the risk of stroke by 76% (age and sex adjusted hazard ratio 1.76, 95% CI 1.31 to 2.37). Stratification for sex showed that the increased risk was only found in men (hazard ratio for men 2.53, 95% CI 1.68 to 3.81, hazard ratio for women 1.27, 95% CI 0.82 to 1.96). After adjusting for cardiovascular risk factors at baseline, the risk remained significantly increased in men (hazard ratio for stroke 2.13, 95% CI 1.35 to 3.36). Subtyping of strokes revealed that unrecognized myocardial infarction was particularly associated with cortical ischemic strokes (hazard ratio for men 3.57, 95% CI 1.79 to 7.12).

Conclusions: Men with unrecognized myocardial infarction have an increased risk of stroke.

INTRODUCTION

Vascular disease outside the brain, such as clinically recognized myocardial infarction (MI), is associated with an increased risk of stroke.[1-3] However, a large proportion of MI in the elderly is unrecognized and is identified only by EKG.[4,5] Various studies have shown that between 21 and 68% of MI are unrecognized and that this proportion is higher in women than in men.[6-14] The prevalence in the general elderly population varies between 1.2 and 6.4%.[4, 12, 15] With respect to the prognosis of unrecognized MI, most studies show an increased risk of new cardiac events and mortality of the same magnitude as the risk increase associated with recognized MI.[6-9] One study reported that men with unrecognized MI even had a 60 to 70% higher risk of death than men with recognized MI.[10] However, few data are present for subsequent non-cardiac events, e.g. stroke. Only the Framingham Study reported that in persons with unrecognized MI stroke occurred at a two to fivefold increased rate versus persons without MI.[6] Thus far, no study focused on the various subtypes of stroke. We examined the relationship between unrecognized MI and incident stroke and its subtypes in the Rotterdam Study, a large population-based cohort study.

METHODS

Study population

The Rotterdam Study is a prospective population-based study of 7,983 participants (aged 55 years and over) from Ommoord, a district of Rotterdam, The Netherlands. The study aims to investigate determinants and causes of chronic diseases in the elderly, including cerebrovascular disease.[16] The study was approved by the Medical Ethics Committee of the Erasmus University. Participants gave written informed consent to participate in the study and to obtain information from treating physicians. At baseline (1990-1993) participants were interviewed and underwent physical examination and blood sampling.[16] At baseline we assessed prior stroke by asking "Did you ever suffer a stroke, diagnosed by a physician?". Medical records of persons who answered 'yes' were checked and a previous stroke was considered present if medical records confirmed it. These persons were excluded from our study, leaving 7,717 participants without stroke at baseline.

Assessment of myocardial infarction

At baseline participants were asked the following questions: "Did you ever experience a heart attack?" and if so, "At what age?", "Who made the diagnosis?" and "Were you admitted to a hospital?". Afterwards a 12-lead EKG was recorded with an ACTA-electrocardiograph (Esaote, Florence, Italy) with a sampling frequency of 500 Hz. All EKGs were processed by the Modular EKG Analysis System (MEANS)[17] to obtain EKG measurement and interpretation. To determine MI MEANS uses a comprehensive set of criteria that partly derive from the Minnesota codes.[18] The MEANS program has been extensively evaluated previously.[17, 19] Of persons with EKG evidence of MI but without self-report, information from GPs and cardiologists was collected to confirm that no clinically manifest MI had occurred. Additional information was also collected of persons with self-reported MI without EKG evidence of MI. This was done to distinguish persons who had suffered a non-Q-wave MI or whose Q-wave had disappeared over time, from persons who mistook other symptoms for MI.

Based on this procedure at baseline, we classified participants as follows. Recognized MI (n=442) included persons with self-reported MI confirmed by matching EKG characteristics or clinical data. Unrecognized MI (n=361) were all participants without documented or self-reported MI, but with EKG characteristics matching an MI. The non-MI reference group (n=5,636) consisted of all persons without indication of MI on EKG and no self-report or medical documentation of an earlier MI.

Of the 7,717 participants without prior stroke, digital EKG data were unavailable for 1,278 persons, mostly due to technical difficulties or insufficient personnel to operate the EKG-apparatus. This left a total of 6,439 participants in the present study.

Assessment of stroke

Assessment and subtyping of strokes in the Rotterdam Study have been extensively described.[1, 20] In summary, after entrance into the Rotterdam Study, participants were continuously monitored for major events through automated linkage of the study database with files from general practitioners and the municipality. Also, nursery home physicians' files and files from general practitioners of participants who moved out of the district were reviewed. For reported events, additional information (including neuroimaging) was obtained from hospital records. Research physicians discussed information on all potential strokes and TIAs with an experienced stroke neurologist (P.J.K.) to verify all diagnoses. This was done blinded for presence of MI. Subarachnoid hemorrhages and retinal strokes were excluded. Follow-up was completed until January 1st, 2002. All strokes were subsequently classified as ischemic, hemorrhagic or unspecified as follows: A stroke was classified as ischemic when a patient had typical symptoms and a CT or MRI scan performed within 4 weeks after the stroke ruled out other diagnoses, or when indirect evidence (deficit limited to 1 limb or completely resolved within 72 hours, atrial fibrillation in absence of anticoagulants) pointed at an ischemic nature of the stroke. A stroke was classified as hemorrhagic when a relevant hemorrhage was shown on CT or MRI scan, or when the participant lost consciousness permanently or died within hours after onset of focal signs. If a stroke did not match these criteria, it was classified as unspecified. Neuroimaging was available for 65% of all events. Although some strokes could be classified based on clinical information only, most strokes without neuroimaging were categorized as unspecified. Of all ischemic stroke events 92% had undergone neuroimaging; for hemorrhagic strokes this was 81%.

Ischemic strokes were further subdivided into clinical syndromes. A hemispheric lacunar ischemic stroke syndrome was diagnosed when a patient suffered from a pure motor stroke, pure sensory stroke, ataxic hemiparesis, or dysarthria and a clumsy hand or arm, in the absence of cortical symptoms or signs (dysphasia, hemineglect, apraxia, acalculia, dysgraphia, or visual field defects). A hemispheric cortical ischemic stroke syndrome was diagnosed when any cortical symptom or sign was present. Ischemic strokes that did not fit into either category remained unspecified. Also, possible posterior fossa ischemic strokes were not analyzed separately, because of too small numbers (n=43).

Possible confounders

A computerized questionnaire was used to obtain information on current health status and medical history at baseline. Smoking status was verified during the baseline interview and participants were classified into one of three categories: current smoker, former smoker, or never smoker. At the research center, sitting blood pressure was measured on the right upper arm using a random-zero sphygmomanometer. In the analyses the average of two measurements, measured at one occasion, was used. Diabetes mellitus was defined as random or post-load serum glucose level exceeding 11.1 mmol/l or the use of oral blood glucose

lowering drugs or insulin. Atrial fibrillation was assessed on an electrocardiogram. Ankle-arm pressure index was measured as described previously.[21] Non-fasting blood samples were taken and serum total cholesterol and high-density lipoprotein cholesterol (HDL) were determined by means of an automated enzymatic procedure. Use of cardiovascular drugs was assessed by interview and pharmacy records. These drugs included nitrates, antihypertensives, statins and antithrombotic agents, including aspirin.

In the analyses, we adjusted for baseline values of these potential confounders.

Data analysis

We used Cox' proportional hazards regression model, adjusted for age and sex, to assess the relationship between MI and risk of stroke, which included ischemic strokes, hemorrhagic strokes and unspecified strokes. Subsequently, we investigated ischemic stroke and its subtypes separately. Persons without MI were taken as reference group. We added an interaction term sex*unrecognized MI to the model to investigate differences between the sexes in prognosis after unrecognized MI. To assess the impact of adjustment for cardiovascular risk factors, we additionally adjusted for systolic blood pressure, diastolic blood pressure, smoking, total and HDL cholesterol, atrial fibrillation, diabetes mellitus, ankle-arm pressure index and use of cardiovascular drugs. Results are presented as hazard ratios (HR) with 95% CI.

RESULTS

Table 1 shows the baseline characteristics of the study population. During 52,915 person-years of follow-up (mean 8.2 years) 505 (213 in men) strokes occurred, of which 299 (133 in men) were ischemic strokes, 48 hemorrhages (20 in men) and 158 unspecified. Of the

Table 1. Baseline characteristics of the study population. Values are percentages or means (SD).

	Without MI n=5,636	Unrecognized MI n=361	Recognized MI n=442
Women (%)	62.0	56.0	31.4
Age (yr)	68.3 (8.5)	72.1 (8.8)	71.1 (8.0)
Present smoker (%)	22.6	27.4	21.0
Former smoker (%)	40.9	38.2	60.2
Diabetes mellitus (%)	9.2	15.2	15.8
Systolic blood pressure (mmHg)	139 (22)	145 (20)	135 (23)
Diastolic blood pressure (mmHg)	74 (11)	75 (12)	70 (11)
Total cholesterol (mmol/l)	6.64 (1.21)	6.53 (1.31)	6.55 (1.20)
HDL-cholesterol (mmol/l)	1.37 (0.36)	1.31 (0.34)	1.16 (0.30)
Atrial fibrillation (%)	2.3	5.3	3.9
Ankle-arm pressure index	1.07 (0.22)	1.00 (0.26)	1.02 (0.27)
Use of cardiovascular drugs (%)	32.8	38.2	83.3

Table 2. Hazard ratios (95% CI) for stroke in persons with unrecognized or recognized MI compared to persons without MI. Adjusted for age and – if applicable – sex.

	Total		Men		Women	
	No. of strokes/n	HR (95%CI)	No. of strokes/n	HR (95%CI)	No. of strokes/n	HR (95%CI)
Without MI	409/5,636	1.00 (ref)	155/2,144	1.00 (ref)	254/3,492	1.00 (ref)
Unrecognized MI	49/361	1.76 (1.31-2.37)	27/159	2.53 (1.68-3.81)	22/202	1.27 (0.82-1.96)
Recognized MI	47/442	1.35 (0.99-1.83)	31/303	1.37 (0.93-2.02)	16/139	1.36 (0.82-2.26)

ischemic strokes 149 (65 in men) were cortical and 74 (32 in men) lacunar; the remaining ischemic strokes were either posterior fossa strokes (n=43) or unspecified.

Table 2 shows the age and sex adjusted HR for stroke. Persons with unrecognized MI at baseline had an almost doubled risk of stroke (HR 1.76, 95%CI 1.31 to 2.37); for persons with recognized MI the risk was lower and only borderline significantly increased. Stratification for sex showed that the increased risk of stroke after unrecognized MI was confined to men (HR in men 2.53, 95%CI 1.68 to 3.81; HR in women 1.27, 95%CI 0.82 to 1.96). The p-value was 0.017 for interaction between sex and unrecognized MI. The risk of stroke after recognized MI was similar for men and women. Figure 1 shows Kaplan-Meier curves for these relationships.

Men had a significantly increased risk of ischemic stroke and particularly cortical ischemic stroke after either a recognized or an unrecognized MI (table 3). The risk increase was however much higher after an unrecognized than a recognized MI. In women, neither recognized nor unrecognized MI was related to the risk of ischemic stroke or ischemic stroke subtypes (table 3).

Additional adjustment for cardiovascular covariates slightly diminished the risk estimates, yet the risk of stroke associated with unrecognized MI remained significantly increased in men (table 4). Analyzing the various cardiovascular drugs separately did not change the results in any way.

DISCUSSION

We found that unrecognized MI is associated with a strongly increased risk of stroke in men, but not in women. In contrast, recognized MI was only borderline associated with an increased risk of stroke after adjustment for vascular covariates. The risk increase was largest for ischemic strokes, particularly cortical ischemic strokes.

Strengths of our study include a mean follow-up of about 8 years, a large sample size, and standardized ascertainment of MI and stroke. Another strength is that our stringent stroke monitoring procedures allowed us to also include stroke patients who were not referred to a hospital, for example persons in nursery homes or persons suffering fatal strokes. Therefore, loss to follow-up was minimal. A disadvantage

Table 3. Age-adjusted hazard ratios (95% CI) for ischemic stroke and subtypes in persons with unrecognized or recognized MI compared to persons without MI, in strata of sex.

	All ischemic stroke		Lacunar ischemic stroke		Cortical ischemic stroke	
	Men	Women	Men	Women	Men	Women
	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
Without MI	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Unrecognized MI	3.22 (1.96-5.28)	1.18 (0.64-2.18)	1.87 (0.56-6.21)	0.86 (0.21-3.59)	3.57 (1.79-7.12)	1.21 (0.53-2.80)
Recognized MI	1.84 (1.16-2.91)	1.12 (0.53-2.41)	1.21 (0.42-3.48)	0.66 (0.09-4.80)	1.97 (1.04-3.75)	1.19 (0.43-3.28)

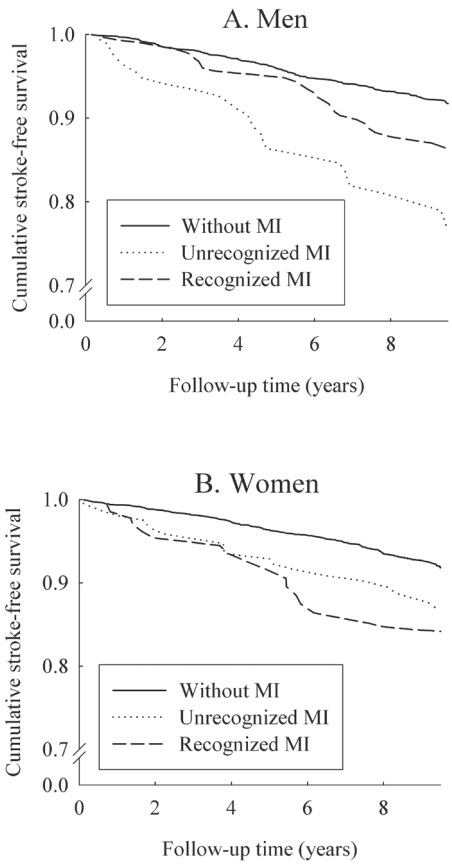

Figure 1. Kaplan-Meier curves for stroke-free survival in persons with unrecognized, recognized and no myocardial infarction, stratified for sex. A: men. B: women

is that in these cases neuroimaging was often lacking (65% of all stroke cases had neuroimaging) and clinical information was not thorough enough to subclassify 158 strokes into ischemic or hemorrhagic. These strokes were thus classified as unspecified. However, although persons suffering unspecified strokes were older than persons with a specified stroke, these groups did not differ from each other in percentage women or the prevalence of unrecognized or recognized MI. Therefore, this could not have led to bias in our analyses. Moreover, the proportion of unspecified strokes in our study is similar to other population-based[22] or even hospital-based[23] studies. Most importantly, because the physicians making the diagnosis of stroke were blinded for presence of MI, any resulting misclassification is likely to have been non-differential

Table 4. Multivariable adjusted hazard ratios (95% CI) for all stroke and ischemic stroke in persons with unrecognized or recognized MI compared to persons without MI, in strata of sex.

	All stroke		Ischemic stroke	
	Men	Women	Men	Women
	HR* (95%CI)	HR* (95%CI)	HR* (95%CI)	HR* (95%CI)
Without MI	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Unrecognized MI	2.13 (1.35-3.36)	1.25 (0.77-2.04)	2.87 (1.66-4.96)	1.35 (0.70-2.60)
Recognized MI	1.37 (0.86-2.19)	1.36 (0.75-2.46)	1.76 (1.00-3.08)	1.01 (0.41-2.51)

* Adjusted for age, systolic blood pressure, diastolic blood pressure, smoking, total and HDL cholesterol, atrial fibrillation, diabetes mellitus, ankle-arm pressure index and use of cardiovascular drugs.

and would result in an underestimation of the effect. Another possible limitation of our study is that EKG data were missing in 1,278 persons. Because persons were invited in random order to the research center, this could not have biased our results.

We focused on stroke, whereas most other studies investigating the prognosis of unrecognized MI did not evaluate stroke separately.[6-10] We found that men with unrecognized MI have a higher risk of stroke than men with recognized MI. These data are in accordance with studies that showed an increased risk of cardiovascular morbidity and mortality in men with unrecognized MI,[6, 10] and with the finding in the Framingham Study of an 1.5 fold increased risk of stroke in men with unrecognized MI versus men with recognized MI.[6]

The important question is why unrecognized MI would be associated with a much higher risk of stroke than recognized MI. A possible explanation is that lack of preventive treatment and specific lifestyle advice contributed to a poorer prognosis after unrecognized MI. Support for this explanation comes from the observation that at baseline the proportions of former smokers and cardiovascular drug users were much higher among persons with a recognized than an unrecognized MI (table 1).

Another explanation would be that men with recognized MI are more likely to die, e.g. of a new MI, while men with unrecognized MI live longer and remain longer at risk for stroke. However, in our dataset the age-adjusted hazard ratios for all mortality (except fatal stroke) and for MI prior to stroke were not significantly increased in men with unrecognized versus recognized MI (HR for mortality, except stroke 1.12 (95% CI 0.83 to 1.51); HR for new MI 0.82 (95% CI 0.45 to 1.48)).

In contrast to our findings in men, we did not find an increased risk of stroke in women with unrecognized MI. This was in accordance with observations in the Framingham study.[6] An explanation for the difference between men and women with unrecognized MI might be that men suffered a clinical stroke, whereas women suffered an asymptomatic or unrecognized stroke. This may have led to underestimation of the risk of stroke in women with unrecognized MI. Indeed, the prevalence and incidence of asymptomatic or unrecognized strokes are reportedly higher in women than in men.[24, 25] Studies investigating unrecognized MI in relation to both clinical strokes and asymptomatic strokes are needed to elucidate this issue.

The risk was particularly increased for cortical ischemic strokes and not for lacunar ischemic strokes. This probably reflects a different etiology of these subtypes of stroke. A substantial proportion of cortical strokes is considered to have a cardiogenic cause, whereas lacunar strokes are more often caused by small vessel disease.[24] Furthermore, a large proportion of lacunar strokes remains clinically asymptomatic and therefore are not reflected in our analyses.[25]

After adjusting for cardiovascular risk factors, the risk of stroke and ischemic stroke remained increased in men with unrecognized MI. There are several explanations for this. Firstly, unrecognized MI may give a better indication of the cardiovascular damage that has accumulated over time than cardiovascular risk factors measured at baseline. Secondly, unrecognized MI may cause global ventricular dysfunction, which may lead to formation of thrombi that become a source of emboli causing stroke.[26] Finally, residual confounding might be present in the following ways: it is possible that unrecognized MI reflect cardiovascular risk factors that we did not measure at baseline, such as genetic risk factors. Additionally, because we adjusted for baseline values of measured risk factors, change in risk factors during follow-up will not be accounted for. Moreover, measurement of risk factors might be imprecise.

At baseline, we did not report presence of unrecognized MI to participants or general practitioners. The decision reflected the perception at that time that an unrecognized MI was less severe than a recognized MI, and was motivated by lack of evidence that treatment after an unrecognized MI could effectively reduce the risk of subsequent cardiovascular events.[6, 10, 27] Our study therefore adequately reflects the situation in the general elderly population where a large proportion of MI remains unrecognized and hence untreated. More recently in the Rotterdam Study, we have started to report findings of unrecognized MI to the participants and their general practitioners. Whether and to what extent this has affected the prognosis of unrecognized MI remains to be seen.

In our study, presence of unrecognized MI in men was particularly related to cortical ischemic strokes that produce large neurological deficits. This suggests that screening the elderly for unrecognized MI using EKG can contribute to identifying persons at increased risk of stroke. Given that EKG-systems are readily available and EKG measurements are easily obtained, whereas their interpretation might be facilitated by computer software,[15] EKGs could be incorporated in prevention programs for stroke. For example, men with unrecognized MI identified in this way, might benefit from installment of preventive treatment and life-style advice. However, it remains to be investigated whether screening for unrecognized MI would be cost-effective.

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Chapter 2.3.2

Unrecognized Myocardial Infarction in relation to Risk of Dementia and Cerebral Small Vessel Disease

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ABSTRACT

Background and purpose: Men, but not women, with an unrecognized myocardial infarction (MI) have an increased risk of cardiac events and stroke compared with those without MI or with recognized MI. We investigated whether unrecognized MI is also a risk factor for dementia and cerebral small vessel disease (white matter lesions (WML) and brain infarction) in two population-based cohort studies.

Methods: In the Rotterdam Study 6,347 participants were classified at baseline (1990-1993) into those with recognized MI (subdivided in Q-wave and non-Q-wave MI), unrecognized MI and without MI based on electrocardiography and interview, and were followed for incident dementia (n=613) until January 1st 2005. In the Rotterdam Scan Study, 436 non-demented persons were similarly classified based on electrocardiography and interview, and underwent brain MR-imaging for the assessment of WML and brain infarction.

Results: In men, unrecognized MI was associated with an increased risk of dementia (compared with men without MI hazard ratio 2.14 (95%CI 1.37-3.35)), and with more WML and more often brain infarction on MRI. In women, no associations were found with unrecognized MI. Recognized MI was not associated with the risk of dementia in either sex. Men, but not women, with recognized MI had more often any brain infarction or asymptomatic brain infarction, especially if they had had a Q-wave MI. No consistent associations were found between recognized Q-wave or non-Q-wave MI and severity of WML. Additional adjustment for cardiovascular risk factors did not change the results.

Conclusions: Men with unrecognized MI have an increased risk of dementia and more cerebral small vessel disease.

INTRODUCTION

In the elderly, between 21 to 68% of all myocardial infarctions (MI) that can be identified by electrocardiography (EKG), are asymptomatic or remain clinically unrecognized.[1,2] Men with an unrecognized MI have an increased risk of clinical cardiac disease when compared with men without MI,[3-5] and even when compared with men with recognized MI.[6] For women, no such differences have been reported. In line with the findings regarding cardiac disease, we found that men, but not women, with unrecognized MI also have an increased risk of clinical stroke compared with persons without MI or recognized MI.[7] However, most cerebrovascular disease in the elderly occurs subclinically as small vessel disease, which can be visualized with magnetic resonance imaging (MRI) as white matter lesions (WML) and asymptomatic (lacunar) brain infarcts.[8,9] Cerebral small vessel disease may be clinically asymptomatic, but is not innocuous, as it is related to an increased risk of clinical stroke,[10] cognitive decline and dementia.[11-13] Whether unrecognized MI is related to cerebral small vessel disease is unknown.

Markers of vascular disease and vascular risk factors have been implicated in the etiology of dementia as well.[14,15] Few studies investigated whether recognized MI was associated with an increased risk of dementia, and those that did reported inconsistent results.[16,17] No study thus far investigated unrecognized MI in relation to risk of dementia.

We hypothesized that unrecognized MI might be an important risk factor for both cerebral small vessel disease and dementia. Therefore, we investigated the association of unrecognized MI with dementia in the Rotterdam Study and with cerebral small vessel disease in the Rotterdam Scan Study. Furthermore, given the unexplained yet consistent finding of an especially unfavorable prognosis of unrecognized MI in men, we also examined whether these associations were different between men and women.

METHODS

Study populations

The Rotterdam Study is a prospective population-based cohort study of 7,983 participants (aged 55 years and over) from Ommoord, a district of Rotterdam, The Netherlands.[18] The study aims to investigate determinants and causes of chronic diseases in the elderly, including dementia. Participants gave written informed consent to participate in the study and to obtain information from treating physicians. At baseline (1990-1993) participants were interviewed and underwent physical examination and blood sampling. We excluded persons, who were not cognitively screened or were demented at baseline, which left a total of 7,046 persons eligible for the present study. Digitized EKGs were obtained in 6,347 persons.

In 1995-1996, 563 participants from the Rotterdam Study, who were still non-demented and who were randomly selected by sex and 5-year age strata, participated in the Rotterdam Scan Study.[19] In these participants a separate interview, physical examination, blood sampling was repeated in 1995-1996, and they also underwent a brain MRI scan. The MRI scan was acquired at the most two weeks after the repeat interview and examination. In 436 of these persons we obtained a digitized EKG.

In both studies, interviews and examinations were held independently from spouse or other family members. Missing EKGs in both studies were random and due to technical problems or too few personnel to operate the apparatus. Both the Rotterdam Study and the Rotterdam Scan Study have been approved by the Medical Ethics Committee of the Erasmus Medical Center, The Netherlands.

Assessment of myocardial infarction on EKG

Assessment of MI was done as reported previously[7] and similarly for both the Rotterdam Study and Rotterdam Scan Study. Participants were asked the following questions: "Did you ever experience a heart attack?" and if so, "At what age?", "Who made the diagnosis?" and "Were you admitted to a hospital?". Afterwards a 12-lead EKG was recorded with an ACTA-electrocardiograph (Esaote, Florence, Italy) with a sampling frequency of 500 Hz. All EKGs were processed by the Modular EKG Analysis System (MEANS) to obtain EKG measurement and interpretation.[20] MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat, using template matching techniques.[20] To determine MI, MEANS uses a comprehensive set of criteria that partly derive from the Minnesota codes.[21] Pathological Q-waves are central in the diagnosis of MI using MEANS. The MEANS program has been extensively evaluated previously.[22-24] Of persons with EKG evidence of MI but without self-report, information from GPs and cardiologists was collected to confirm that no clinically manifest MI had occurred. Additional information was also collected of persons with self-reported MI without EKG evidence of MI. This was

done to distinguish persons who had suffered a non-Q-wave MI or whose Q-wave had disappeared over time, from persons who mistook other symptoms for MI.

Based on this procedure, we classified participants as follows. Recognized Q-wave MI included persons with self-reported MI confirmed by matching EKG characteristics. Recognized non-Q-wave MI included persons with self-reported MI confirmed only by clinical data. Unrecognized MI were all participants without documented or self-reported MI, but with EKG characteristics matching an MI. All unrecognized MI were therefore Q-wave MI. The non-MI reference group consisted of all persons without indication of MI on EKG and no self-report or medical documentation of an earlier MI.

Ascertainment of incident dementia

In the Rotterdam Study the diagnosis of incident dementia was made following a three-step protocol.[25] At baseline (1990-1993) and during three follow-up visits (1993-1994, 1997-1999, 2002-2004) two brief tests of cognition (Mini-Mental State Examination (MMSE) and Geriatric Mental State schedule (GMS) organic level) were used to screen all subjects. Screen-positives (MMSE score < 26 or GMS organic level > 0) underwent the Cambridge examination for mental disorders of the elderly (Camdex). Persons who were suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for diagnosis. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. The diagnosis of dementia and major subtypes of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III-R), Alzheimer's disease (NINCDS-ADRDA), and vascular dementia (NINDS-AIREN) by a panel of a neurologist, neuropsychologist and research physician. Follow-up was complete until January 1st, 2005.

MRI procedures

Within the Rotterdam Scan Study, cranial MRI scanning was performed in all participants with a 1.5-T scanner (VISION-MR, Siemens, Erlangen, Germany) using standard T1, T2 and proton-density weighted MR sequences. MRI acquisition parameters have been described.[9, 26]

We obtained continuous volumetric measures of WML (expressed as percentage of intracranial volume to correct for individual head-size differences) using validated automated image analysis.[26] We defined brain infarction on MRI as focal hyperintensities on T2-weighted images, 3 mm in size or larger. Proton-density scans were used to distinguish infarctions from dilated perivascular spaces. Hyperintensities in the white matter also had to have corresponding prominent hypointensities on T1-weighted images, in order to distinguish them from WML. History of stroke and TIA was assessed by self-report and by checking

medical records, independent from MRI data. We defined asymptomatic brain infarctions as evidence of one or more infarctions on MRI without a history of corresponding TIA or stroke. Intrarater agreement for detection of infarcts was good (κ=0.80).[26]

Assessment of covariables

In both the Rotterdam Study and the Rotterdam Scan Study physical examinations were performed using the same protocol and computerized questionnaires were used to obtain information on current health status and medical history. Smoking status was verified during the interview. Sitting blood pressure was measured on the right upper arm using a random-zero sphygmomanometer. We used the average of two measurements, measured at one occasion. Diabetes mellitus was defined as random or post-load serum glucose level exceeding 11.1 mmol/l or the use of oral blood glucose lowering drugs or insulin. Carotid intima-media thickness was measured by longitudinal 2-dimensional ultrasound of the carotid artery. We calculated the mean common carotid artery intima-media thickness as the mean of 4 locations: the near and far wall of both the right and left common carotid artery. Atrial fibrillation was assessed on an EKG. Serum total cholesterol and high-density lipoprotein cholesterol (HDL) were determined by means of an automated enzymatic procedure in non-fasting blood samples. Use of cardiovascular drugs was assessed by interview and pharmacy records. These drugs included nitrates, antihypertensives, statins and antithrombotic agents, including aspirin. Genotyping of *APOE* was performed on coded DNA specimens without knowledge of the outcomes.

Statistical analysis

We tested differences in baseline demographic covariables between the three groups using Student's t-test for continuous variables and χ^2-test for dichotomous variables.

In the Rotterdam Study we assessed the association of recognized Q-wave MI, recognized non-Q-wave MI, and unrecognized MI with risk of dementia and major subtypes of dementia with Cox' proportional hazard models, adjusted for age and sex. Additionally we adjusted for cardiovascular risk factors. Because previous reports suggested a difference in prognosis of unrecognized MI between men and women,[5,7] we subsequently examined the association between MI and dementia in men and women separately and computed an interaction term between MI and sex. To assess whether the association between MI and dementia was mediated by stroke, we repeated the analyses excluding persons with prevalent stroke and censoring those with incident stroke at time of stroke.

In the Rotterdam Scan Study we used general linear models to calculate mean WML volume in persons with no MI, unrecognized MI, and recognized MI (Q-wave and non-Q-wave MI separately). We used logistic regression models to calculate odds ratios for brain infarction in persons with recognized or unrecognized MI compared with persons without

MI. All analyses were adjusted for age and sex and subsequently stratified by sex. Additionally, we adjusted for cardiovascular risk factors.

RESULTS

Tables 1 and 2 show the characteristics of the study population. In the Rotterdam Study, 424 participants (297 in men) had had a recognized MI, of whom 197 (130 in men) had a non-Q-wave MI. Of the 345 persons with unrecognized MI, 159 were men. In the Rotterdam Scan Study 40 persons (32 in men) had had a recognized MI, of whom 24 (19 in men) had a non-Q-wave MI. Twenty-two persons (6 in men) had had an unrecognized MI. In both studies there were no significant differences in baseline characteristics between persons with a recognized Q-wave MI and those with a recognized non-Q-wave MI. In both studies persons with recognized Q-wave MI or non Q-wave MI used more often cardiovascular drugs than those with unrecognized MI or without MI. Moreover, in the Rotterdam Study persons with unrecognized MI had a higher blood pressure and were more often smokers than persons without MI or with recognized Q-wave or non-Q-wave MI.

Table 1. Characteristics of the study population at baseline of the Rotterdam Study.

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

Values are percentages or means (standard deviation)

* Significantly different (p-value <0.05) from persons without MI (age and sex adjusted, if applicable)

† Significantly different (p-value <0.05) from persons with recognized MI (age and sex adjusted, if applicable)

‡ This group includes both persons with a Q-wave MI and a non-Q-wave MI. No significant differences were present between these two subgroups

§ This group included only persons with Q-wave MI

Incident dementia

During 58,712 person years of follow-up in the Rotterdam Study we identified 613 dementia patients, of whom 479 were diagnosed with Alzheimer's disease, 71 with vascular dementia

Table 2. Characteristics of the study population of the Rotterdam Scan Study.			
	No MI	Recognized MI‡	Unrecognized MI§
N	374	40	22
Age (yrs)	72.9 (8.0)	74.3 (7.1)	74.6 (8.1)
Women %	52.7	20.0*	72.7†
Presence APOE ε4 allele %	31.6	31.6	23.5
Body mass index (kg/m^2)	26.2 (3.6)	26.5 (3.0)	26.2 (3.5)
Current smokers %	18.0	12.5	4.5
Systolic blood pressure (mmHg)	145.3 (20.9)	145.5 (20.6)	147.5 (20.7)
Diastolic blood pressure (mmHg)	76.5 (11.6)	73.2 (10.7)	77.7 (9.5)
Cholesterol (mmol/l)	5.9 (1.0)	5.9 (1.0)	5.8 (1.1)
HDL-cholesterol (mmol/l)	1.3 (0.4)	1.0 (0.2)*	1.3 (0.4)†
Intima media thickness (mm)	0.86 (0.14)	0.95 (0.18)*	0.84 (0.13)†
Diabetes mellitus %	4.0	10.0	4.5
Atrial fibrillation %	2.1	2.5	9.1
Use of cardiovascular drugs %	43.9	97.5*	72.7*†

Values are percentages or means (standard deviation)

* Significantly different (p-value <0.05) from persons without MI (age and sex adjusted, if applicable)

† Significantly different (p-value <0.05) from persons with recognized MI (age and sex adjusted, if applicable)

‡ This group includes both persons with a Q-wave MI and a non-Q-wave MI. No significant differences were present between these two subgroups

§ This group included only persons with Q-wave MI

and 63 with dementia due to other causes. The incidence rate of dementia among persons without MI was 9.95 per 1,000 person-years; among those with recognized Q-wave MI this was 10.28 per 1,000 person-years and among those with recognized non-Q-wave MI 15.85 per 1,000 person-years; finally, among persons with unrecognized MI the incidence rate was 16.40 per 1,000 person-years.

Table 3 shows that unrecognized MI was associated with a more than doubled risk of dementia, but only in men. In men, unrecognized MI was associated with an increased risk of both Alzheimer's disease and vascular dementia: age-adjusted hazard ratios (95% confidence interval (CI)) were 2.53 (1.49-4.30) for Alzheimer's disease (126 cases) and 2.03 (0.71-5.80) for vascular dementia (37 cases). Recognized MI was not significantly associated with the risk of dementia (table 3). The p-value of the interaction term between unrecognized MI and sex was <0.01, between recognized Q-wave MI and sex 0.42, and between recognized non-Q-wave MI and sex 0.28. Additional adjustment for cardiovascular risk factors did not change the estimates (table 3). Excluding previous stroke cases and censoring incident stroke cases at time of stroke did not attenuate the estimates either; if anything the association became stronger: age-adjusted hazard ratio (95% CI) for dementia in men with unrecognized MI was 2.33 (1.38-3.95).

Table 3. The association between myocardial infarction and dementia by sex.

	Total			Men			Women		
	n/N	HR (95% CI)*	HR (95% CI)†	n/N	HR (95% CI)*	HR (95% CI)†	n/N	HR (95% CI)*	HR (95% CI)†
No MI	524/5,578	1.00(ref)	1.00(ref)	157/2,154	1.00(ref)	1.00(ref)	367/3,424	1.00(ref)	1.00(ref)
Recognized MI	43/424	1.06 (0.78-1.46)	1.12 (0.77-1.64)	20/297	0.87 (0.55-1.39)	0.89 (0.51-1.54)	23/127	1.35 (0.88-2.06)	1.58 (0.94-2.65)
Q-wave MI	*18/227*	*0.83 (0.52-1.33)*	*0.96 (0.56-2.13)*	*9/167*	*0.71 (0.36-1.39)*	*0.79 (0.36-1.70)*	*9/60*	*1.01 (0.52-1.99)*	*1.48 (0.69-3.19)*
non-Q-wave MI	*25/197*	*1.34 (0.89-2.00)*	*1.30 (0.79-2.13)*	*11/130*	*1.07 (0.58-1.98)*	*1.00 (0.48-2.07)*	*14/67*	*1.69 (0.99-2.89)*	*1.66 (0.85-3.25)*
Unrecognized MI	46/345	1.22 (0.90-1.65)	1.35 (0.95-1.92)	22/159	2.14 (1.37-3.35)	2.23 (1.24-4.01)	24/186	0.87 (0.58-1.32)	1.17 (0.74-1.83)

n: number of dementia cases, N: total number of persons, HR: hazard ratio, CI: confidence interval

* adjusted for age and sex (if applicable)

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

Table 4. The association between myocardial infarction and brain infarction by sex.

	OR (95% CI) for any brain infarction			OR (95% CI) for asymptomatic brain infarction		
	Total	Men	Women	Total	Men	Women
No MI	1.00(ref)	1.00(ref)	1.00(ref)	1.00(ref)	1.00(ref)	1.00(ref)
Recognized MI	3.57 (1.74-7.34)	3.50 (1.54-7.96)	4.33 (0.92-20.28)	3.25 (1.49-7.09)	3.35 (1.39-8.07)	3.32 (0.60-18.51)
Q-wave MI	*6.39 (2.13-19.16)*	*6.98 (2.06-23.70)*	*4.63 (0.38-57.11)*	*5.41 (1.63-17.96)*	*6.50 (1.75-24.15)*	*2.30 (0.12-45.20)*
non-Q-wave MI	*2.41 (0.97-5.99)*	*2.13 (0.75-6.11)*	*4.17 (0.61-28.60)*	*2.39 (0.91-6.28)*	*2.17 (0.72-6.60)*	*3.95 (0.50-31.03)*
Unrecognized MI	2.36 (0.93-5.97)	7.19 (1.17-44.07)	1.51 (0.49-4.66)	2.43 (0.93-6.34)	6.49 (0.93-45.29)	1.74 (0.56-5.38)

Values are odds-ratios (OR) with 95% confidence intervals (CI), adjusted for age and sex (if applicable)

MRI outcomes

In the Rotterdam Scan Study, we found that men with unrecognized MI had on average more WML than men without MI (figure 1). Volume of WML did not differ between men with recognized Q-wave or non-Q-wave MI and men without MI. In contrast, women with unrecognized MI had an average WML load that was similar to that of women without MI. Women with a recognized Q-wave MI had on average more WML than women without MI. However, the interaction term between unrecognized MI and sex was significant (p=0.02), whereas the interaction term between recognized Q-wave MI and sex was not (p=0.07).

After adjustment for cardiovascular risk factors, the results were slightly attenuated, but the difference in volume of WML between men with unrecognized MI and without MI remained significant (fully adjusted difference in WML volume 1.18% (95%CI 0.08-2.29)).

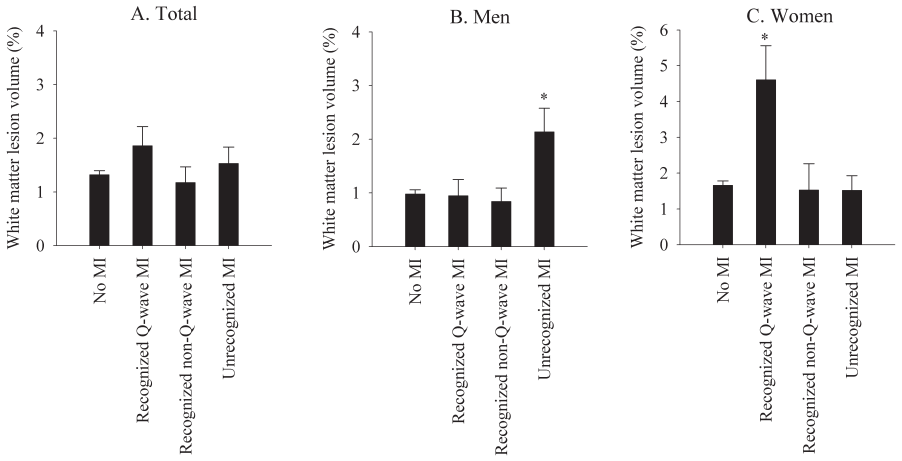

Figure 1. Association between myocardial infarction and volume of white matter lesions (n=436). Volume is expressed as percentage of intra-cranial volume to adjust for head-size differences. Bars represent means, adjusted for age and sex (if applicable); lines represent standard errors. * significantly different from persons without MI (p<0.05).

Of the 374 persons without MI 90 had a brain infarction on MRI (75 of these were asymptomatic). Among the 16 persons with recognized Q-wave MI 10 had a brain infarction (7 asymptomatic). Among the 24 persons with recognized non-Q-wave MI 10 had a brain infarction (8 asymptomatic). Finally, among the 22 persons with unrecognized MI 10 had a brain infarction (9 asymptomatic). Men with a recognized MI were more likely to have any brain infarction or an asymptomatic brain infarction on their MRI scan than men without MI, especially if they had had a recognized Q-wave MI (odds ratio 6.98 (95% CI 2.06 to 23.70); table 4). Likewise, the prevalence of any brain infarctions was more than 7-fold increased in men with unrecognized MI (table 4). Women with a recognized MI had a non-significantly increased prevalence of brain infarction on MRI, similarly for Q-wave and non-Q-wave MI. In contrast, unrecognized MI was not associated with the presence of

brain infarction in women. The p-values of the interaction term between unrecognized MI and sex were 0.15 for any brain infarction and 0.25 for asymptomatic brain infarction. The corresponding p-values of the interaction term between recognized Q-wave MI and sex were 0.77 for any brain infarction and 0.53 for asymptomatic brain infarction; and between recognized non-Q-wave MI and sex 0.55 for any brain infarction and 0.62 for asymptomatic brain infarction. Adjusting for cardiovascular risk factors did not change the associations. If anything, the odds-ratio of brain infarction associated with unrecognized MI became stronger in men (full-adjusted odds ratio 8.79 (95% CI 1.06-73.17)).

DISCUSSION

We found that men, but not women, with unrecognized MI had an increased risk of dementia, more WML, and more often any brain infarction or an asymptomatic brain infarction compared with those without MI, even when known cardiovascular risk factors were accounted for. Recognized MI, both Q-wave and non-Q-wave, was not associated with the risk of dementia in either sex. Men, but not women, with recognized MI had more often any brain infarction or an asymptomatic brain infarction on MRI, especially if they had had a Q-wave MI. No consistent associations were found between recognized Q-wave or non-Q-wave MI and severity of WML.

The strengths of our studies include the population-based setting, the large number of participants, and the virtually complete follow-up for dementia. Moreover, we focused on clinical and subclinical manifestations of both cardiac and cerebrovascular disease. A limitation is the cross-sectional study design of the Rotterdam Scan Study, which might limit our interpretation of the data with respect to the temporal relationship between MI and MRI parameters. Another possible limitation could be that MI on EKG was diagnosed using the MEANS computer program, which might have led to misclassification. However, this program has been extensively validated and diagnoses correlate well with diagnoses made by an experienced cardiologist.[20, 23] Also, any misclassification is likely to be non-differential since MEANS diagnoses were made independent from clinical diagnosis of dementia and assessment of MRI outcomes.

Most studies reporting on the prognosis of unrecognized MI did not investigate neurological outcomes, but focused on cardiac events.[3, 4] Only the Rotterdam Study and the Framingham study have looked at clinical stroke separately.[5, 7] In the present study we focused on dementia and cerebral small vessel disease.

Our observation regarding the association between unrecognized MI and subclinical cerebral small vessel disease is in line with our previous report with respect to clinical stroke.[7] Indeed, both clinical and subclinical cerebrovascular disease have been shown to be closely associated with each other[10, 27, 28] and to share similar (cardiovascular) risk factors.[29]

In turn, our finding that persons with unrecognized MI have an increased risk of dementia fits well with previous studies reporting that markers of vascular disease and vascular risk factors are involved in the pathogenesis of dementia,[14, 15] presumably by leading to subclinical cerebral small vessel disease.[30, 31]

Moreover, we found that the increased risk was confined to men, and not women. This is in accordance with studies that showed an increased risk of cardiovascular morbidity and clinical stroke in men with unrecognized MI, but not in women.[5, 7] An explanation for this difference between men and women might be the higher background prevalence of cerebrovascular disease in men compared with women. Another possibility is that misclassification of MI on EKG may occur more often in women. EKG abnormalities that can be mistaken for MI, but are not caused by coronary disease are more often seen in women than in men, possibly caused by difficulties in correctly placing the electrodes due to breast tissue.[32] Because of this possible non-differential misclassification of our determinant, dilution of the effect might have occurred and therefore the true effect may have been missed in women in our dataset. Such misclassification might also explain the higher prevalence of unrecognized MI among women than men. Finally, to rule out the possibility that sex differences occurred by chance, other studies should seek to replicate our findings.

In contrast to our findings in persons with unrecognized MI, we did not find consistent associations of recognized MI, neither Q-wave nor non-Q-wave MI with dementia, WML or brain infarction, for either sex. We only found statistically significant associations for recognized Q-wave MI with any brain infarction or an asymptomatic brain infarction in men and with WML in women. This is in line with published data on differences between unrecognized and recognized MI with respect to prognosis for cardiovascular morbidity.[6, 7] A possible explanation for this difference is that inherent lack of preventive treatment and specific lifestyle advice contributed to a poorer prognosis after unrecognized MI. Support for this comes from the observation that in both the Rotterdam Study and Rotterdam Scan Study the proportions of cardiovascular drug users were higher among persons with a recognized than with an unrecognized MI. Still, adjusting for cardiovascular risk factors did not change the associations. This may indicate that unrecognized MI gives a better indication of the cardiovascular damage that has accumulated over time than cardiovascular risk factors measured only once at baseline.

In conclusion, our study shows that presence of unrecognized MI is associated with an increased risk of dementia and a higher prevalence of cerebral small vessel disease in men, but not in women. Given the large proportion of MI that remain unrecognized in the general elderly population,[1] our data suggest that screening men for unrecognized MI using EKG might identify those at an increased risk of various adverse outcomes. These persons could then benefit from subsequent installment of preventive therapy. However, before such screening is initiated, our results first need to be replicated in other population-based studies.

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Chapter 2.4

Total Cerebral Blood Flow in relation to Cognitive Function: The Rotterdam Scan Study

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ABSTRACT

Cerebral hypoperfusion has been associated with worse cognitive function. We investigated the association between cerebral blood flow and cognition and whether this association is independent of brain volume. In 892 participants aged 60-91 years of the population-based Rotterdam Scan Study, we measured total cerebral blood flow (tCBF) and brain volume using MRI. Lower tCBF was associated with worse information processing speed, executive function and global cognition. However, after correcting tCBF for brain volume, these associations disappeared. The association between tCBF and cognition may be mediated or confounded by brain atrophy. Future studies on tCBF should take into account brain atrophy.

INTRODUCTION

Elderly persons often suffer from deterioration of cognitive function. Vascular risk factors may contribute to cognitive impairment by affecting blood flow to the brain.[1] Moreover, it has been suggested that cerebral hypoperfusion precedes and possibly contributes to onset of clinical dementia.[2] To assess perfusion at the brain tissue level is difficult as most measurement techniques are invasive and complex. Phase-contrast magnetic resonance imaging (MRI) enables fast and accurate measurement of total cerebral blood flow (tCBF) and has shown to be applicable in population-based studies.[3] Previous studies showed that lower tCBF assessed with phase-contrast MRI was related to poorer cognition, in particular information processing speed, and dementia.[4, 5] However, these studies did not assess whether this association was independent of brain atrophy. It can be hypothesized that smaller brain volume leads to decreased cerebral metabolic demand, and as such confounds the association between cerebral blood flow and cognitive function.

Thus, the aim of our study was to investigate whether diminished cerebral blood flow is associated with specific domains of cognitive function independent of brain volume.

METHODS

Participants

This study is embedded within the Rotterdam Study, a large population-based cohort study in the Netherlands.[6] The original study population consisted of 7,983 participants aged 55 years and older from the Ommoord area, a suburb of Rotterdam. In 2000, the cohort was expanded with 3,011 persons (≥ 55 years).[6] From August 2005 to May 2006, we randomly selected 1,073 members of this cohort expansion for participation in the Rotterdam Scan Study, a population based brain-imaging study. After exclusion of individuals who were demented or had MRI contraindications 975 persons were eligible, of whom 907 participated and gave written informed consent. Due to physical inabilities (e.g. back pain), imaging could not be performed or completed in 12 individuals. Therefore, a total of 895 complete MR examinations were performed. The institutional review board approved the study.

MRI scan protocol

MRI of the brain was performed on a 1.5-Tesla MRI scanner (General Electric Healthcare, Milwaukee, WI, USA), using an 8-channel head coil. For flow measurement, 2D phase-contrast imaging was performed as described previously.[7] In brief, a sagittal 2D phase-contrast MRI angiographic scout image was performed. On this scout image, a transverse imaging plane perpendicular both to the precavernous portion of the internal carotid arteries and to the middle part of the basilar artery was chosen for a 2D gradient-echo phase-contrast sequence (repetition time= 20ms, echo time= 4 ms, field-of-view= 19 cm^2, matrix= 256x160, flip angle= 8°, number of excitations= 8, bandwidth= 22.73 kHz, velocity encoding= 120 cm/s, slice thickness= 5 mm). For an example, see (Vernooij et al. 2007). Acquisition time was 51 seconds, and no cardiac gating was performed.[3] We further performed three high-resolution axial MRI sequences, i.e. a T1 weighted sequence, a proton-density weighted sequence, and a fluid attenuated inversion recovery (FLAIR) sequence.[7]

Measurement of tCBF and total brain perfusion

Flow was calculated from the phase-contrast images using Interactive Data Language (IDL)-based custom software (Cinetool version 4, General Electric Healthcare, Milwaukee, WI, USA).[7] Two independent experienced technicians drew all manual regions of interest (ROI) drawing and performed subsequent flow measurements (inter rater correlations (n=533) > 0.94 for all vessels).[7] In three persons, tCBF could not be measured due to incorrect positioning of the phase-contrast imaging plane, leaving a total of 892 persons in our analysis.

We calculated total brain perfusion (in ml/min per 100 ml) by dividing tCBF (ml/min) by each individual's brain volume (ml) and multiplying the obtained result by 100.[7]

Assessment of brain volume

For the assessment of brain volume, the structural MRI scans (T1-weighted, PD-weighted, FLAIR) were transferred to a Linux workstation. Preprocessing steps and the classification algorithm have been described elsewhere.[8] In summary, preprocessing included coregistration, nonuniformity correction and variance scaling. We used the k-nearest neighbor classifier[9] to classify scans into brain tissue and cerebrospinal fluid using the multispectral MR intensities. All segmentation results were visually inspected and if needed manually corrected. To remove non-cerebral tissue, e.g. eyes, skull, and cerebellum, we applied nonrigid registration[10] to register to each brain a template scan in which these tissues were manually masked. Brain volume was calculated by summing all voxels across the whole brain, to yield volumes in ml.

Cognitive function

Cognitive function was assessed with a neuropsychological test battery comprising the Mini-Mental State Examination (MMSE), the Stroop test, the Letter-Digit Substitution Task (LDST)(number of correct digits in one minute), the Word Fluency Test (WFT) (animal categories), and a 15-Word Verbal Learning Test (15-WLT) (based on Rey's recall of words).[11] For each participant z-scores were calculated for each test separately (individual test score minus mean test score divided by the standard deviation), except for MMSE. To obtain more robust measures, we constructed compound scores for information processing speed, executive function, memory and global cognitive function. The compound score for information processing speed was the average of the z-scores for the Stroop reading and Stroop color naming subtask and the LDST. Executive function included the z-scores of the Stroop interference subtask, the LDST and the WFT (number of animals in one minute). The compound score for memory was the average of the z-scores for the immediate and delayed recall of the 15-WLT. For global cognitive function we used the average of the z-scores of the Stroop test (average of the reading, color naming and interference subtask), the LDST, the WFT, and the immediate and delayed recall of the 15-WLT.[11]

Covariates

We assessed the level of education and current smoking by interview. Systolic and diastolic blood pressures were measured twice on the right arm with a random-zero sphygmomanometer. The mean of the two readings was used in the analyses. Diabetes mellitus was defined as the use of blood glucose-lowering medication or fasting serum glucose level ≥ 7.0 mmol/l. Carotid plaque score was assessed by Doppler ultrasound.[12]

Data analysis

We evaluated the association of both tCBF (ml/min) and total brain perfusion (ml/min per 100 ml brain tissue) per standard deviation (SD) increase with cognitive function using

multiple linear regression models. All analyses were adjusted for age, sex and education. To examine whether associations were independent of vascular risk factors, we additionally adjusted for current smoking, systolic and diastolic blood pressure, diabetes mellitus and carotid plaque score.

RESULTS

Characteristics of the study population are shown in Table 1. Lower tCBF was associated with worse performance on tests of information processing speed, executive function and global cognition, but not with MMSE score and memory performance (Table 2).

Total brain volume was a strong determinant of tCBF (per SD increase in brain volume 36.00 ml/min increase in tCBF; 95% confidence interval 30.00; 42.10). The associations of tCBF with cognition disappeared upon correcting for brain volume (Table 2). Adjustments for vascular risk factors did not change any of these associations (Table 2).

Table 1. Characteristics of the Study Population.

Characteristics	Participants (n=892)
Men, n	441 (49.4)
Age, years	67.5 (5.5)
Primary education, n	38 (4.4 %)
Systolic Blood Pressure, mmHg	143.8 (18.5)
Diastolic Blood Pressure, mmHg	81.0 (10.2)
Diabetes Mellitus, n	85 (9.6 %)
Current Smokers, n	267 (29.9 %)
Plaques in Carotid Artery, range 0-12*	3.0 (1.0-5.0)
Mini Mental State Examination, score	27.9 (1.8)
Brain Volume, ml	976.8 (114)
Total Cerebral Blood Flow, ml/min	497.4 (86.2)
Total Brain Perfusion, ml/min/100ml brain tissue	51.2 (8.8)

Values are means (SD) or numbers (percentages)
* median, interquartile range

DISCUSSION

We found that persons with a low tCBF performed significantly worse on tasks assessing information processing speed, executive function and global cognitive function compared with persons with higher tCBF. However, total brain perfusion, indicating the flow in ml per 100 ml of brain tissue volume, was not associated with cognitive function. Adjustments for vascular risk factors did not change the results.

Table 2. Association of Total Cerebral Blood Flow (tCBF) and Total Brain Perfusion with Cognitive Function (z-scores), using Linear Regression Models (n=892).

	Difference in test scores (95% CI) per standard deviation (SD) increase in flow measure.				
	MMSE	Z-score information processing speed	Z-score executive function	Z-score memory	Z-score global cognition
tCBF					
Model 1	0.08 (-0.04;0.19)	0.08 (0.03;0.14)	0.07 (0.02;0.12)	0.00 (-0.07;0.06)	0.05 (0.01;0.10)
Model 2	0.09 (-0.03;0.20)	0.07 (0.02;0.13)	0.06 (0.01;0.11)	0.00 (-0.07;0.06)	0.05 (0.01;0.10)
Total Brain Perfusion					
Model 1	0.07 (-0.05;0.19)	0.04 (-0.02;0.09)	0.00 (-0.05;0.05)	0.03 (-0.04;0.09)	0.02 (-0.02;0.07)
Model 2	0.08 (-0.04;0.20)	0.04 (-0.02;0.09)	0.00 (-0.05;0.05)	0.03 (-0.03;0.09)	0.02 (-0.02;0.07)

Model 1 = adjusted for age, sex and level of education

Model 2 = additionally adjusted for systolic blood pressure, diastolic blood pressure, current smoking, diabetes mellitus and plaque score

Before interpreting the results, some methodological issues need to be addressed.The strengths of our study are its population-based setting, the high response rate and the large sample size. A limitation is the cross-sectional design, which restricts our interpretation of the data with respect to cause and consequence. Furthermore, we only assessed average brain perfusion. Hence, we cannot exclude that brain perfusion in distinct brain regions may relate differently to cognitive performance. Finally, we could not measure blood flow into the cerebellum as we measured blood flow in the basilar artery at the level after the anterior and posterior inferior cerebellar arteries arise.

It can be hypothesized that cerebral hypoperfusion causes brain atrophy that subsequently leads to cognitive decline.[1, 13] On the other hand, it may also be that because of a diminished demand, brain atrophy itself affects cerebral blood flow. Thus, the association between tCBF and cognitive function may be mediated or confounded by brain atrophy.

In the past, CBF velocity measured by Transcranial Doppler ultrasonography has been used as a proxy measure for cerebral blood flow. Several studies using CBF velocity reported that subjects with greater CBF velocity were less likely to have dementia.[2] Furthermore, a greater CBF velocity was found to be related with larger hippocampal and amygdalar volumes.[2]

More recently, associations of tCBF with speed, executive function[4] and dementia[5] were found using phase-contrast MRI. Our data are in line with these studies, since we also found the strongest associations for cognitive domains of speed and executive function.[4, 5] However, none of those previous studies assessed whether the associations between cerebral blood flow and cognitive function were independent of brain volume. We

went a step further by correcting for brain volume, and found no associations between total brain perfusion and cognitive function.

Thus far, only a few small studies reported that regional patterns of hypoperfusion in the brain may relate to cognitive decline or dementia independent of global differences.[14, 15] As mentioned, we could not evaluate this in our study. Further studies are needed to investigate this.

In conclusion, our findings show that the relation between total cerebral blood flow and worse performance on several domains of cognitive function is dependent on brain volume.

Our study emphasizes that future studies on tCBF should take into account brain atrophy.

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Chapter 2.5

The Role of Retinal Vascular Caliber in Brain Atrophy on MRI

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ABSTRACT

A relation between vascular disease and brain atrophy has been suggested, but evidence remains limited. Retinal vessels may reflect the condition of intracerebral vessels. Particularly, wider retinal venular diameters are associated with cerebrovascular disease. We investigated whether retinal vessel diameters are related to brain atrophy in 683 elderly persons (mean age 67.0 and 52% women). Retinal arteriolar and venular diameters were semi-automatically measured on digitized fundus transparencies. Using automated quantification of MRI-scans we obtained whole-brain volume and volumes of grey matter, white matter, and white matter lesions (WML). Brain infarcts were rated visually. Both arteriolar and venular diameters were associated with whole brain volume. However, venular diameter but not arteriolar diameter was associated with white matter volume. This association was independent from cardiovascular risk factors. Arteriolar diameter was associated with WML, but this attenuated after adjustment. No associations were found with grey matter volume or brain infarcts. Our data provide further evidence that vascular pathology as reflected by wider venular diameter is associated with brain atrophy, particularly white matter atrophy.

INTRODUCTION

Cerebral small vessel disease (white matter lesions (WML) and brain infarcts) and cardiovascular risk factors have been associated with whole-brain atrophy.[1-3] Studies investigating grey matter (GM) and white matter (WM) separately suggested that these associations were stronger for WM atrophy than GM atrophy.[4, 5]

Retinal vessels may reflect the condition of the intracerebral vessels.[6-8] Whereas previous studies reported on the ratio of retinal arterioles to venules (AV-ratio), it is now increasingly being recognized that arterioles and venules differ with respect to determinants and outcomes.[9-12] Narrower arterioles are related to blood pressure and hypertension, while wider venules are associated with atherosclerosis, hypoxia, inflammation, and also stroke.[11, 13-17] This distinct pattern between arterioles and venules was further confirmed by a recent study showing smaller arteriolar diameter to be associated with poorer large vessel compliance and larger venular diameter with poorer compliance of small vessels.[18]

Previously, a lower AV-ratio has also been related with visually rated whole-brain atrophy scores.[19] However, arteriolar and venular diameters have not been studied separately. Moreover, that previous study did not investigate GM and WM separately.

We investigated how retinal arteriolar and venular diameters are related to volumetric measures of whole-brain atrophy and WM and GM atrophy separately.

METHODS

Study population

The Rotterdam Study is a population-based cohort study ongoing since 1990.[20] The cohort was expanded in 2000-2002 with 3,011 persons (≥ 55 years), who underwent a follow-up examination, including eye examinations, in 2002-2004. Between August 2005 and May 2006, we randomly invited 1,073 of the 3,011 persons for participation in the Rotterdam Scan Study. The institutional review board approved the study. We excluded persons who were demented (as assessed by a three-step protocol[21]) or who had MRI contraindications. This left 975 eligible persons, of whom 907 participated and gave written informed consent. Complete and usable MRI examinations were available in 871 persons; missing datasets were mostly due to claustrophobia or motion artifacts. Eye examinations including fundus transparencies were available in 775 of those 871 participants. After additional exclusion of 92 subjects with ungradable fundus transparencies on both eyes, a sample of 683 persons remained for the current study. The mean duration between the eye examinations and the MRI scan was 1.0 year (SD 0.5 years).

Assessment of retinal vessels

Fundus transparencies were taken centered on the optic disc after pharmacological mydriasis, and digitized with a high-resolution scanner.[14] For each participant the qualitatively best digitized image of either eye was analyzed with the Retinal Vessel Measurement System.[22] Retinal arteriolar and venular diameters were measured and summarized using Parr-Hubbard-Knudtson formulas and Littmann's formula to adjust for refractive errors of the eye.[11] Two trained graders performed the assessments, masked to participants' clinical characteristics. Pearson's correlation coefficients for intergrader agreement were 0.87 for arteriolar and 0.91 for venular diameters. Intragrader agreement ranged from 0.65 to 0.86.[14]

Assessment of brain parameters

Brain MRI was performed on a 1.5-Tesla scanner and included T1 weighted, proton-density weighted, and FLAIR sequences. Imaging parameters and classification algorithms have been described elsewhere.[5, 14] In summary, we used the k-nearest neighbor classifier to classify scans into cerebrospinal fluid, GM, normal WM, and WML. Validation results against manual segmentations have been reported and were very good to excellent.[5] We removed non-cerebral tissue with validated non-rigid registration[23] to register to each brain a template scan in which these tissues were manually masked.

Lacunar infarcts were rated visually and defined as lesions ≥ 3 mm in size exhibiting the same signal characteristics as cerebrospinal fluid on all sequences, and, if located supratentorially, with a hyperintense rim on the FLAIR sequence. Cortical infarcts were those infarcts showing involvement of cortical gray matter.[5, 24]

Assessment of covariates

We used the following cardiovascular risk factors measured in 2002-2004 as possible confounders: smoking status (current, former, or never), blood pressure, atherosclerotic plaques in the carotid artery, body mass index, fasting serum total and HDL cholesterol, leukocyte count, diabetes mellitus, and arteriolar oxygen saturation. Details on the assessment of these covariates have been described.[14]

Statistical analysis

All volumes were expressed as percentage of intra-cranial volume to correct for head-size. Whole-brain volume was defined as intra-cranial volume minus cerebrospinal fluid volume. Total WM was defined as normal WM plus WML. WML were further natural log transformed because of skewness of the untransformed measure.

To enable better comparison between the effects of retinal vessels on different tissue types we calculated z-scores for each tissue type separately (z-score = (tissue volume - mean tissue volume) / SD). We investigated the association of retinal vessels with brain tissue volumes and WML volume using linear regression models and with lacunar infarcts using logistic regression models.

All analyses were adjusted for age, sex, and following recent recommendations[9] the other vessel diameter (analyses of venular diameter adjusted for arteriolar diameter and vice versa). Next, we adjusted for cardiovascular risk factors. Subsequently, we redid all analyses after excluding persons with a cortical infarct on MRI (n=16).

RESULTS

The table shows the characteristics of persons with gradable and no or ungradable fundus transparencies. Persons with no or non-gradable transparancies were significantly older than those with good quality transparencies, but no other differences were present between the two groups. Seventy-six persons had a brain infarct on MRI, of whom 60 had lacunar infarcts only. The Figure shows the associations between retinal vessel diameters and brain tissue volumes. Both narrower arterioles and wider venules were associated with smaller whole-brain volume. For narrower arterioles this was due to both smaller GM and WM volumes, but not statistically significantly. In contrast, for wider venules the smaller brain volume was not due to smaller GM volume, but instead significantly due to smaller (normal and total) WM volume. Finally, narrower arteriolar diameter was significantly associated with larger WML volume.

Adjusting for cardiovascular risk factors hardly changed the associations of larger venular diameter with normal WM (–0.08 (95%CI –0.17;0.01)) and total WM (–0.08 (95%CI

–0.17;0.01)). However, the association of arterioler diameter with WML attenuated (–0.05 (95%CI –0.14;0.04)).

No associations were seen between retinal vessel diameters and any brain infarct (odds-ratio per SD increase in arteriolar diameter: 0.98 (95%CI 0.73;1.32); odds-ratio per SD increase in venular diameter: 1.02 (95%CI 0.76;1.37)).

Excluding persons with cortical infarcts did not change any of the associations.

Table. Characteristics of the study population

Variable	Gradable transparencies n = 683	No or non-gradable transparencies n = 188
Age, years	67.0 (5.0)	69.0 (6.5) *
Women, n	357 (52%)	86 (46%)
Carotid plaques, score	2.80 (2.65)	3.39 (2.99)
Systolic blood pressure, mmHg	143 (18)	146 (20)
Diastolic blood pressure, mmHg	81 (10)	81 (10)
Total cholesterol, mmol/l	5.7 (0.9)	5.7 (1.0)
High density lipoprotein cholesterol, mmol/l	1.4 (0.4)	1.4 (0.4)
Diabetes, n	59 (9%)	25 (13%)
Body mass index, kg/m^2	27.5 (3.9)	27.6 (3.1)
Current smoking, n	199 (29%)	59 (31%)
Past smoking, n	275 (40%)	79 (42%)
Leukocyte count, 10^9/l	6.7 (1.8)	6.6 (1.5)
Arterial oxygen saturation, %	96.7 (1.2)	96.6 (1.1)
Arteriolar diameter, µm	149 (15)	NA
Venular diameter, µm	232 (22)	NA

Values are unadjusted means (standard deviation) or number (percentages)

* significantly different from persons with gradable transparencies (sex-adjusted p-value<0.05)

NA: not applicable

DISCUSSION

In this population-based study both narrower arterioles and wider venules were associated with whole-brain atrophy. However, wider venules but not narrower arterioles were associated with WM atrophy, independent from cardiovascular risk factors. Narrower arterioles were associated with more WML, but this association attenuated after adjusting for cardiovascular risk factors.

Strengths of our study include the population-based setting, large sample size and the quantitative assessment of separate brain tissue volumes on MRI. Since participants were from the cohort expansion, no overlap exists with individuals in our previous studies on retinal vessels. A limitation is the cross-sectional study design, which limits the interpretation of the results with respect to cause and effect.

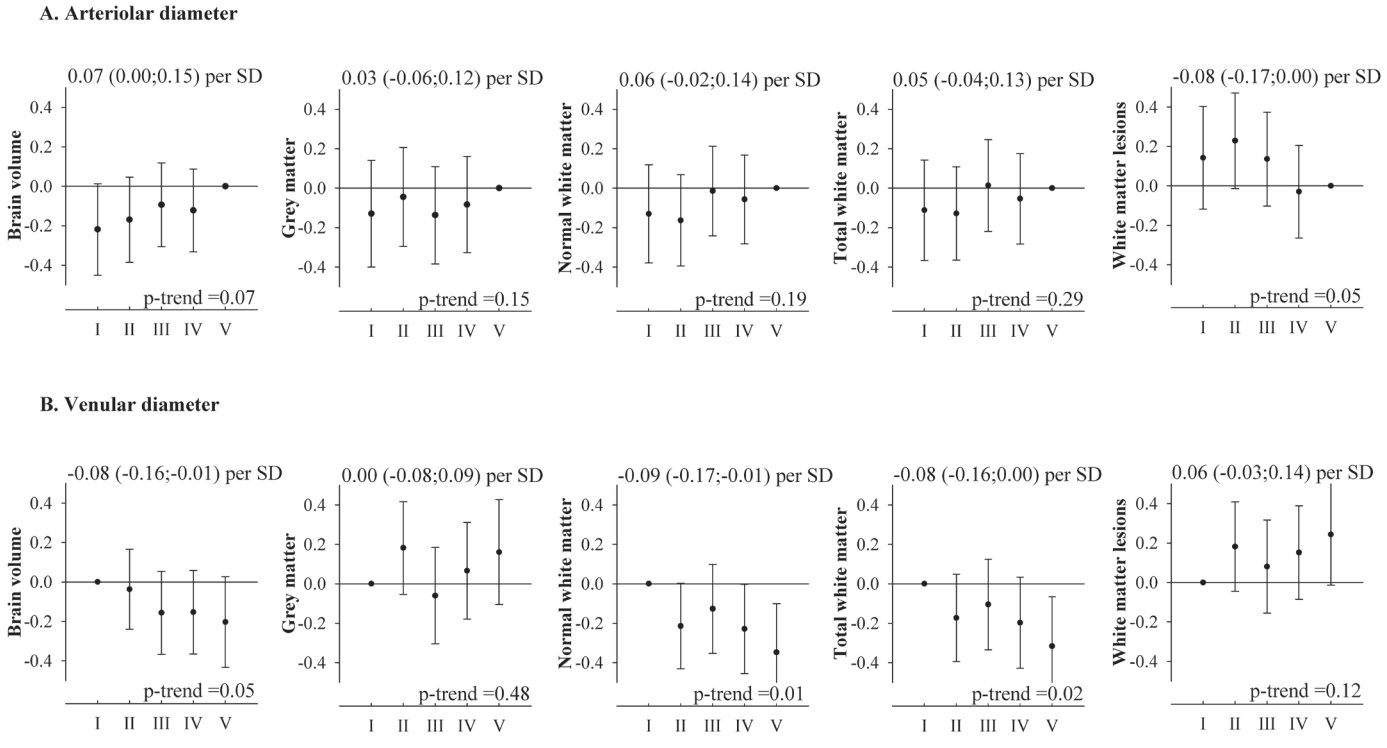

Figure. The association between quintiles of retinal vessel diameters and z-scores of brain tissue volumes.

On the x-axis quintiles of retinal vessel diameters are plotted. On the y-axis z-scores of brain tissue volumes are plotted. Total white matter is the sum of normal white matter and white matter lesions. White matter lesions are further natural log transformed. Dots represent mean difference. Lines represent confidence intervals. Values are adjusted for age, sex, and the other retinal vessel diameter. For arteriolar diameter the highest quintile is taken as reference group (reflecting widest arterioles); for venular diameter the lowest quintile is taken as reference group (reflecting narrowest venules).

At the top of the plots, regression coefficients (with 95% confidence intervals) for the linear models are expressed per standard deviation (SD) increase in the arteriolar or venular diameter.

The p-trend is calculated by modeling the quintiles as a continuous variable in the linear regression.

A. Arteriolar diameter; B. Venular diameter

This report expands on recent findings that retinal arteriolar and venular diameters differ with respect to determinants and clinical correlates.[9, 10, 12, 13, 15, 25] Previously, wider venules were shown to be related with atherosclerosis, hypoxia, and inflammation,[13, 14] which are known risk factors for cerebral small vessel disease. Moreover, whereas narrower arterioles have been associated with large vessel compliance, wider venular diameter correlates with poorer compliance of small vessels.[18] In turn, small vessel disease both in the brain and in other parts of the body is closely associated with WM atrophy.[2, 4, 5] Accordingly, we showed that wider venular diameter was associated with WM atrophy. Therefore, our data provide further evidence that vascular pathology as reflected by wider venular diameter may play a role in the pathogenesis of cerebral small vessel disease and WM atrophy.

We also found that additional adjustment for cardiovascular risk factors only marginally changed the association between venular diameter and WM atrophy. An explanation is that venular diameter is an independent and perhaps better marker of WM atrophy than those risk factors. Another possibility is that venules reflect risk factors for WM atrophy that we did not measure in our study, e.g. genetic factors.

Our study showed an association between arteriolar diameter and WML. This is seemingly in contrast to our previous report showing venular diameters to be related with WML and brain infarcts.[16] However, in our previous report we did not find any association cross-sectionally, but only with progression of WML and brain infarcts in a longitudinal analysis. Indeed, in the current report we also do not find an association cross-sectionally between retinal vessel diameters and brain infarcts. Moreover, the cross-sectional association between arteriolar diameter and WML attenuated after adjustment for cardiovascular risk factors.

Finally, we did not find an association between retinal vessel diameters and GM atrophy, which fits our previous reports suggesting that vascular risk factors are more associated with WM atrophy than GM atrophy.[4, 5]

In conclusion, we found that wider venular diameters are related to WM atrophy, providing further insights into the vascular basis of brain atrophy, particularly WM atrophy, and cerebral small vessel disease.

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Part 3

Chapter 3.1

Brain Tissue Volumes in relation to Cognitive Function and Risk of Dementia

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ABSTRACT

We investigated in a population-based cohort study the association of global and lobar brain tissue volumes with specific cognitive domains and risk of dementia. Participants (n=490;60-90years) were non-demented at baseline (1995-1996). From baseline brain MRI-scans we obtained global and lobar volumes of CSF, GM, normal WM, white matter lesions and hippocampus. We performed neuropsychological testing at baseline to assess information processing speed, executive function, memory function and global cognitive function. Participants were followed for incident dementia until January 1st 2005. Larger volumes of CSF and WML were associated with worse performance on all neuropsychological tests, and an increased risk of dementia. Smaller WM volume was related to poorer information processing speed and executive function. In contrast, smaller GM volume was associated with worse memory function and increased risk of dementia. When investigating lobar GM volumes, we found that hippocampal volume and temporal GM volume were most strongly associated with risk of dementia, even in persons without objective and subjective cognitive deficits at baseline, followed by frontal and parietal GM volumes.

INTRODUCTION

Several biomarkers for cognitive impairment and dementia have been identified using magnetic resonance imaging (MRI) of the brain. Medial temporal lobe atrophy, including hippocampal atrophy, is closely related to memory impairment and is a strong predictor of dementia even in asymptomatic persons.[1-5] Subcortical vascular disease, as reflected by white matter lesions (WML) and lacunar infarcts, is thought to contribute to the development of dementia by primarily affecting a different cognitive domain than memory, namely information processing speed.[6, 7]

Several studies have suggested that persons with whole-brain atrophy also have poorer global cognition and suffer more often from dementia than persons without atrophy.[8, 9] However, little is known whether this applies evenly to atrophy of all brain regions and for all cognitive domains. Moreover, few studies distinguished between grey matter (GM) and white matter (WM) atrophy. Previous studies have used visual ratings of sulcal width as an indirect marker of GM atrophy, and ventricular enlargement as an indirect marker of WM atrophy, and found inconsistent results regarding their relationship with specific cognitive domains.[10-13] Recent advances in the analysis of brain MRI-data have opened the way for automated in vivo volumetric quantification of the whole brain, and of GM and WM.[14, 15] The use of these direct volumetric measures of GM and WM atrophy may allow for a better assessment of specific effects on cognition.

Atrophy of the hippocampus, which is a predominantly GM structure, is thought to be one of the first detectable signs of dementia.[1] Post-mortem and neuroimaging studies in dementia patients have shown that atrophic changes in GM are present throughout the brain, and that these changes probably develop later in the disease course.[16-20] Little is known about whether brain atrophy outside the hippocampus is discernible during the preclinical phase of dementia.

We investigated in a population-based cohort study the association of GM and WM volume with specific cognitive domains and with the risk of dementia. Furthermore, we investigated how atrophy of the different cerebral lobes was related to dementia, and whether lobar atrophy predicted dementia in asymptomatic persons.

METHODS

Participants

This study is based on the Rotterdam Scan Study, a large population-based cohort study in the Netherlands, investigating age-related brain changes on MRI.[6, 21] At baseline (1995-1996), we randomly invited participants (60-90 years) stratified by sex and 5-year age strata from the Zoetermeer Study and the Rotterdam Study to participate in the Rotterdam Scan Study.[6, 22] Individuals who were demented, blind or had an MRI contraindication were excluded from the study. The present study is restricted to participants originating from the Rotterdam Study (n=563), because their scanning protocol included an additional high-resolution MR sequence. All persons gave written informed consent and the study was approved by the medical ethical committee of the Erasmus Medical Center, Rotterdam.

MRI measures at baseline

Brain scans were performed on a 1.5-Tesla MRI System (VISION MR, Siemens AG, Erlangen, Germany). We obtained a proton-density, a T2-weighted, and a high-resolution inversion-recovery double contrast 3D HASTE sequence for our multi-spectral volumetry.[23] Among the 563 participants, 52 developed claustrophobia during MRI acquisition and 21 additional datasets were unusable due to various technical reasons (e.g. excessive motion artifacts) leaving a total of 490 participants in our present study.

Image preprocessing and the tissue classification algorithm have been described elsewhere.[23, 24] Briefly, preprocessing included co-registration, non-uniformity correction and intensity normalization. Afterwards, we used the k-nearest-neighbor classifier[25] to classify voxels into cerebrospinal fluid (CSF), GM, normal WM, and WML. To remove non-cerebral tissue, we used non-rigid transformation[26, 27] to register to each brain a template scan, in which all non-cerebral tissue was manually masked. Validation methods and results have been described and showed very good to excellent agreement between automated classification and manual classification as reference.[23, 24] For an example of the classification result see Ikram et al.[24]

For measurement of lobar brain tissue volumes, we first created a template scan, in which the lobes were labeled according to a slightly modified version of the segmentation protocol as described by Bokde et al.[28, 29] Subsequently, we used a validated non-rigid registration algorithm to map this template to each brain.[26, 27] Figure 1 shows an example of this segmentation, which uses anatomical landmarks and cerebral fissures as boundaries to distinguish the four major lobes (frontal, parietal, occipital, and temporal). By combining this lobar segmentation with the tissue classification algorithm we were able to obtain lobar volumes of GM, normal WM and WML separately.

Hippocampal volumes were manually outlined on coronal HASTE-slices reconstructed perpendicular to the long axis of the hippocampus.[3] Brain infarcts were rated visually as

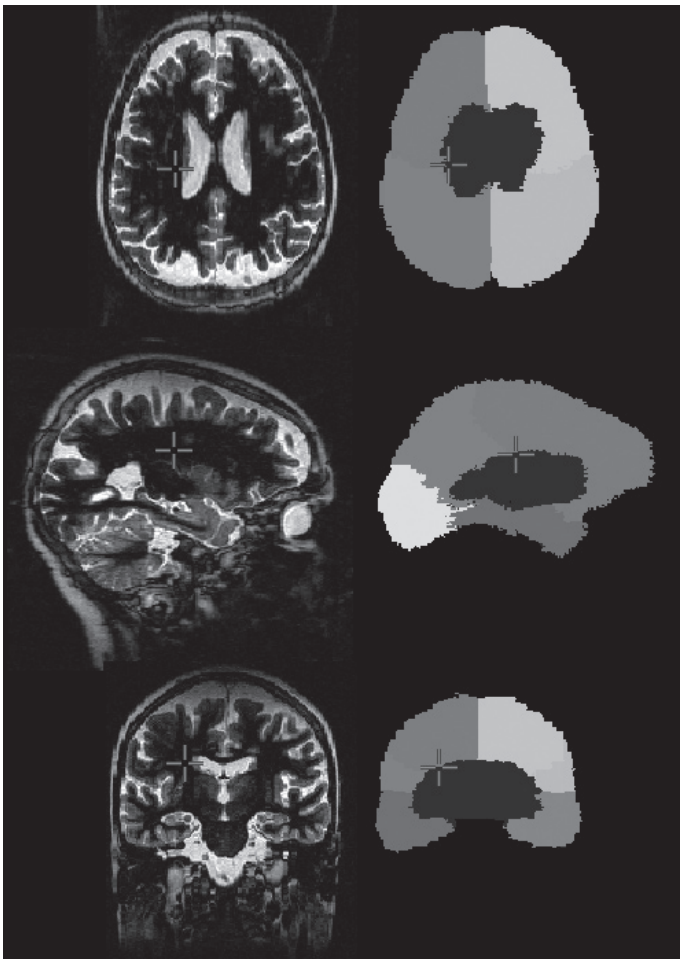

Figure 1. Haste-Odd sequence and the result after segmentation into the various brain lobes using non-rigid registration. Red: left frontal lobe; white: right frontal lobe; dark green: left parietal lobe; light green: right parietal lobe; dark blue: left temporal lobe; light blue: right temporal lobe; yellow: left occipital lobe; purple: central region comprising basal ganglia and corpus callosum. The right occipital lobe is not shown on these cross-sections. See this figure in color in the Appendix.

focal hyperintensities on T2-weighted images, 3 mm in size or larger and with a corresponding prominent hypointensity on T1-weighted images. Intrarater agreement for detection of infarcts was good (κ=0.80).[21]

Cognitive function at baseline

At baseline, participants underwent the following neuropsychological tests: the Mini-Mental State Examination (MMSE),[30] the Stroop test,[31] the Letter-Digit Substitution Task,[32] a verbal fluency task,[33] and a 15-word verbal learning test (based on Rey's recall of words).[34] For each participant, we calculated z-scores for each test separately, except for MMSE (z-score =

test score minus mean test score divided by the standard deviation). To obtain more robust outcome measures for cognition, we used the individual neuropsychological test scores to construct compound scores for information processing speed, for executive function, for memory, and for global cognitive function.[6] The compound score for information processing speed was calculated as the average of the z-scores for the first and second subtask of the Stroop test and the Letter-Digit Substitution Task. The score for executive function was the average of the z-scores for the third subtask of the Stroop test, the Letter-Digit Substitution Task, and the verbal fluency task. The compound score for memory was the average of the z-scores for the immediate and delayed recall of the 15-word verbal learning test. The compound score for global cognitive function was the average of the z-scores for the Stroop test (averaged across the three subtasks), the Letter-Digit Substitution Task, the verbal fluency test, and the immediate and delayed recall of the 15-word verbal learning test.[6]

Memory impairment as measured with neuropsychological tests is the first detectable neuropsychological sign of incipient dementia.[35] Moreover, subjective memory complaints too are thought to be highly predictive of incident dementia.[36] Therefore, we also questioned persons at baseline on subjective memory complaints by asking a single question: "Do you have complaints about your memory performance?" This question has been shown to predict incident dementia.[37]

Ascertainment of incident dementia

Assessment and subtyping of dementia cases in the Rotterdam Scan Study followed the protocol of the Rotterdam Study.[38] We screened all participants for dementia at baseline and at two follow-up examinations (1999-2000, 2001-2002) using a three-step protocol: Two brief tests of cognition (MMSE and Geriatric Mental State schedule (GMS) organic level) were used to screen all participants. Screen-positives (MMSE score < 26 or GMS organic level > 0) underwent the Cambridge examination for mental disorders of the elderly (Camdex).[39] Persons who were suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for the diagnosis.

In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. The diagnosis of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III-R). Follow-up for incident dementia was complete until January 1st 2005.

Covariates

At baseline, information on education and current health status was obtained by interview and physical exam. Smoking status was verified and participants were classified into one

of three categories: current smoker, former smoker or never smoked. Blood pressure was measured twice at the right arm with a random-zero sphygmomanometer. The average of the two values measured at one occasion was used. Hypertension was defined as one of the following: a systolic blood pressure of 160 mm Hg or higher, or a diastolic blood pressure of 100 mm Hg or higher, or current use of blood pressure lowering drugs for the indication of hypertension (Grades 2 and 3 according to the 1999 World Health Organization guidelines).[40] Diabetes mellitus was defined as non-fasting serum glucose level exceeding 11.1 mmol/l or the use of oral blood glucose lowering drugs or insulin. *APOE* genotype was determined in 420 participants.

Statistical analysis

All brain tissue volumes were expressed as percentage of intra-cranial volume (= CSF + GM + normal WM + WML) to correct for individual head-size differences. Therefore, a larger relative volume of CSF indicates a smaller whole-brain volume. Total WM was defined as the sum of normal WM and WML. WML were further natural log transformed because of skewness of the untransformed measure. Because initial analyses did not show any consistent differences between left and right lobar volumes, we summed volumes of both sides for further analyses.

With linear regression we investigated the relationship between global brain tissue volumes and cognitive function. With Cox' proportional hazards model we calculated hazard ratios for dementia per standard deviation increase in global brain tissue volumes. All analyses were adjusted for age, sex, education level, and additionally for cardiovascular risk factors and presence of brain infarcts, and stratified on presence of the *APOE* ε4-allele. In similar analyses we investigated how lobar brain tissue volumes were related to cognitive function and risk of dementia. We performed these analyses per standard deviation (SD) increase in the various volumes in order to be able to compare the magnitude of the effect of these volumes with each other.

Initially, we studied the whole cohort. Subsequently, we investigated whether the relationships between brain tissue volumes and risk of dementia were present even in persons without objective and subjective cognitive problems at baseline. For this, we stepwise excluded persons with increasingly less severe cognitive problems at baseline.[3] We first excluded persons with subjective memory complaints AND z-memory < 1.5 SD of age- and education-specific means (which was calculated by regressing age and education with memory and taking those persons whose standardized residual was lower than 1.5). Secondly, we additionally excluded persons without subjective memory complaints but with z-memory < 1.5 SD of age- and education-specific means. Next, we also excluded persons with z-memory < 1.0 SD of age- and education-specific means. Finally, all persons with

subjective memory complaints OR z-memory < 1.5 of age- and education-specific means were excluded.

RESULTS

Table 1 shows the baseline characteristics of the study population.Table 2 shows the cross-sectional association between global brain tissue volumes and cognitive performance. Larger volumes of CSF and WML were related to a lower MMSE score and all cognitive domains. Larger volumes of total WM and normal WM were related to better performance on the MMSE, higher information processing speed, and borderline with better executive function, whereas larger GM volume was significantly related to better memory function. Investigating lobar brain tissue volumes in relation to cognitive function yielded similar effects for all lobar volumes (data not shown).Adjusting for cardiovascular risk factors and brain infarcts did not change the results.

Table 1. Baseline characteristics of the study population.

N	490
Age, yr	73.4 (7.9)
Women	249 (51)
Primary education only	149 (30)
Hypertension	250 (51)
Diabetes mellitus	24 (5)
Current smoker	87 (18)
Former smoker	264 (54)
APOE ε4 carriers [a]	131 (31)
MMSE, score	27.7 (2.2)
Brain infarcts	137 (28)
Cerebrospinal fluid, %ICV	22.6 (3.7)
Grey matter, %ICV	46.6 (4.1)
Normal white matter, %ICV	29.5 (6.4)
Total white matter, %ICV	30.8 (5.7)
White matter lesions, %ICV [b]	-0.33 (1.26)
Hippocampal volume, %ICV	0.57 (0.08)

Values are means (standard deviation) or numbers (%). MMSE Mini-Mental State Examination; ICV Intra-cranial volume

[a] assessed in 420 persons

[b] natural log transformed

During a mean follow-up of 5.9 years (standard deviation 1.6; range 0.1-9.0 years), 46 persons developed dementia (incidence-rate 16.0 per 1000 person-years). Table 3 shows the risk of dementia associated with global brain tissue volumes. Larger volumes of CSF and WML were related to an increased risk of dementia. Larger GM volume indicated a

Table 2. Global brain tissue volumes and cognitive function.

Brain tissue volume	MMSE	z-score information processing speed	z-score executive function	z-score memory	z-score global cognitive function
Cerebrospinal fluid, %ICV (per SD)	-0.52 (-0.77;-0.27)	-0.22 (-0.31;-0.14)	-0.18 (-0.26;-0.09)	-0.10 (-0.21;0.00)	-0.14 (-0.21;-0.06)
Grey matter, %ICV (per SD)	0.01 (-0.18;0.20)	-0.04 (-0.10;0.03)	0.03 (-0.04;0.09)	0.11 (0.03;0.19)	0.04 (-0.02;0.10)
Total white matter, %ICV (per SD)	0.22 (0.01;0.43)	0.13 (0.06;0.20)	0.06 (-0.01;0.13)	-0.05 (-0.14;0.03)	0.02 (-0.04;0.09)
Normal white matter, %ICV (per SD)	0.32 (0.10;0.54)	0.16 (0.08;0.23)	0.09 (0.02;0.17)	-0.02 (-0.11;0.07)	0.06 (-0.01;0.12)
White matter lesions, %ICV (per SD) [a]	-0.31 (-0.51;-0.10)	-0.11 (-0.18;-0.04)	-0.15 (-0.22;-0.08)	-0.10 (-0.18;-0.02)	-0.12 (-0.18;-0.06)

Values are amount of change (95% confidence interval) in MMSE score or z-score per standard deviation increase in brain tissue volumes, adjusted for age, sex and education. MMSE Mini-Mental State Examination, ICV Intra-cranial volume, SD standard deviation

[a] natural log transformed

decreased risk of dementia, whereas total and normal WM volumes were not related to the risk of dementia.

All relations remained unchanged after adjusting for cardiovascular risk factors and brain infarcts (Table 3). Also, stratification by *APOE* genotype did not alter the results.

Figure 2 shows the association of hippocampus volume and GM volume in various lobes with the risk of dementia. Hippocampus volume was most strongly associated with the development of subsequent dementia, followed by volumes of temporal GM, frontal GM and parietal GM, whereas occipital GM volume was not associated with the risk of dementia. As expected, the more people we excluded from the lower end of the memory performance distribution at baseline the weaker the associations became of hippocampus and GM volumes with the risk of dementia, although the overall pattern in strength of the associations remained unchanged (Figure 2). However, even after excluding persons with z-memory below 1.0 SD of age- and education-specific means at baseline, hippocampus volume and temporal GM volume remained significantly associated with dementia (Figure 2). Only after excluding persons with subjective memory complaints OR z-memory below 1.5 SD (which was 33% of the cohort) did the associations of hippocampus and temporal GM with dementia become statistically non-significant.

Of note is that global GM and temporal GM volumes also included hippocampal volume; however, subtracting hippocampal volume from these volumes did not change the results in any way.

In line with our observations on global volumes of normal and total WM, lobar volumes of WM were not associated with the risk of dementia.

Table 3. Global brain tissue volumes and the risk of dementia.

Brain tissue volume	Risk of dementia HR (95% CI)	
	Model I	Model II
Cerebrospinal fluid, %ICV (per SD)	2.51 (1.56; 4.06)	2.46 (1.51; 4.01)
Grey matter, %ICV (per SD)	0.66 (0.48; 0.91)	0.66 (0.48; 0.91)
Total white matter, %ICV (per SD)	1.02 (0.71; 1.47)	1.04 (0.73; 1.50)
Normal white matter, %ICV (per SD)	0.88 (0.61; 1.27)	0.90 (0.62; 1.30)
White matter lesions, %ICV (per SD) [a]	1.52 (1.02; 2.27)	1.57 (1.03; 2.38)

HR hazard ratios, CI confidence intervals, ICV Intra-cranial volume, SD standard deviation

Model I: adjusted for age, sex and education

Model II: additionally adjusted for diabetes mellitus, smoking, hypertension and brain infarcts

[a] natural log transformed

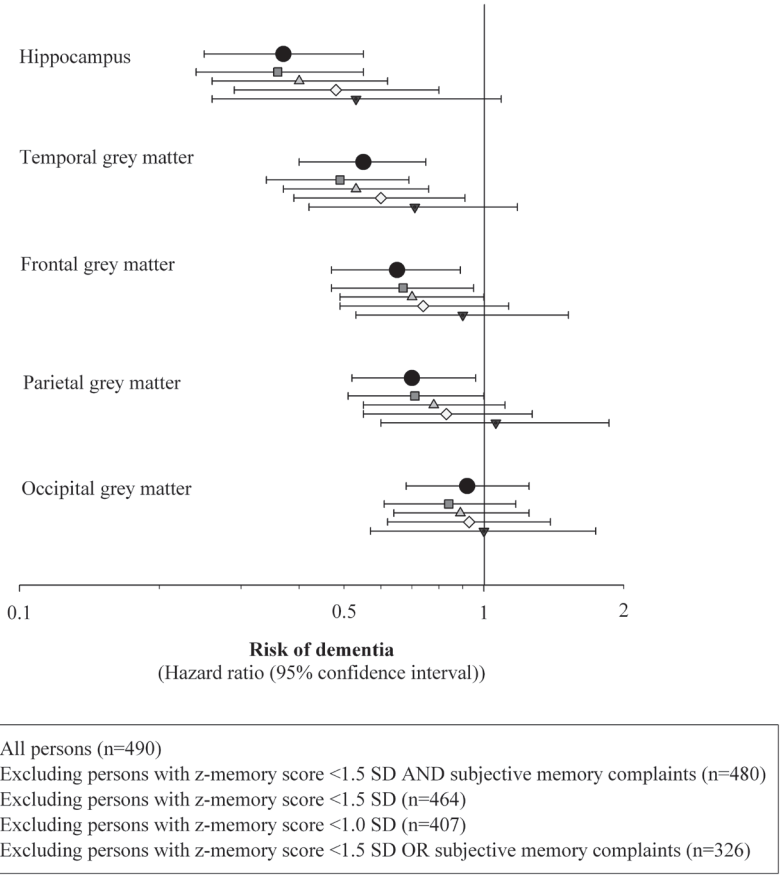

Figure 2. The association of hippocampus volume and grey matter volume in various lobes with the risk of dementia. Symbols represent hazard ratios per standard deviation increase in volume, adjusted for age, sex and education; horizontal error bars represent the 95% confidence interval. Note that the x-axis is depicted on a logarithmic scale. SD standard deviation

DISCUSSION

In this population-based cohort study we found that volumes of WM and GM relate differently to specific cognitive domains and to the risk of dementia. Atrophy of WM was related to worse MMSE scores, lower psychomotor speed and worse executive function, but not to risk of dementia. In contrast, GM atrophy was related to worse memory performance and to an increased risk of dementia. When analyzed at the lobar level, hippocampal and temporal GM atrophy were most strongly associated with dementia, followed by frontal GM atrophy and parietal GM atrophy. Occipital GM atrophy was not associated with risk of dementia. Upon step-wise excluding persons with increasingly less severe objective or subjective cognitive deficits at baseline, this pattern remained, although the effect estimates became smaller and ultimately non-significant.

Strengths of our study include the population-based setting, the large sample size, the volumetric quantification of global and lobar brain tissue volumes, and the long and virtually complete follow-up for dementia. Moreover, by taking into account memory complaints and neuropsychological performance at baseline, we were able to also investigate asymptomatic persons. A possible limitation is that in some cases misclassification in diagnosis or subtyping of dementia could have occurred. However, because the diagnosis and subtyping was made blinded for brain tissue volumes at baseline, any misclassification is likely to be non-differential and will lead to an underestimation of the effect we found. Another consideration is that we focused on selected cognitive domains (i.e. memory, information processing speed, and executive function), but did not investigate other cognitive domains such as visuospatial processing, visuoperceptual tasks, or naming. Moreover, we studied global and lobar tissue volumes but not subcortical tissue volumes or volumes of specific regions within lobes. Future research using a more extensive test battery coupled with more detailed regional volumes might reveal subtle associations that we could not assess in this study.

Thus far, several imaging studies have shown that whole-brain atrophy is related to poorer cognition and increased risk of dementia.[8,9,41] In our dataset, this is reflected in the associations of larger CSF volume with poor cognitive function, and with the risk of dementia. However, we went a step further by making a distinction between WM and GM volumes and found that these had different effects on cognitive performance and dementia.

We found that WM atrophy was related to worse MMSE score and information processing speed. In the cerebral WM, the axonal structures are covered by myelin sheets, which are pivotal in increasing the speed of information transfer.[42] Damage to myelin may therefore lead to poorer performance on tests measuring information processing speed. These my-

elin sheets are usually very susceptible to ischemic damage caused by subcortical vascular disease.[42, 43] Subcortical vascular disease can be seen on structural MRI not only as WML and lacunar infarcts, but also as WM atrophy.[42, 44] Moreover, on diffusion-tensor-imaging (DTI) damage to myelin sheets is reflected in loss of microstructural integrity of WM. Our data using structural MRI are in line with studies using DTI that show that a decline in white matter microstructural integrity is related to lower MMSE score, information processing speed and executive function, but not to memory.[45, 46]

We found that GM atrophy was related to poorer memory performance and an increased risk of dementia. In contrast, Mungas et al.[47, 48] reported that (change in) GM was related to global cognition, executive function and speed, but not to memory. However, that study was based on a heterogeneous study population including cognitively impaired and demented persons, whereas we focused on persons who were non-demented at baseline. Differences in study population and severity of cognitive impairment might therefore explain these seemingly contradictory findings. This is supported by the fact that exclusion of demented persons in the study by Mungas et al.[48] attenuated the relation between decrease in GM and decline in executive function.

Memory impairment is a pivotal symptom of dementia, and both are related to neuronal loss,[49] which can be visualized as GM atrophy on MRI.[50] Previously, we did not find an association between increasing age and global GM atrophy in the general non-demented population.[24] This suggests that GM atrophy is a process specific to those, who are at an increased risk of dementia. When analyzing the separate lobar tissue volumes we found that atrophy of the hippocampus and temporal GM was most strongly associated with dementia, followed by frontal GM atrophy and parietal GM atrophy. This fits well with several imaging studies that have investigated patterns of GM atrophy in persons with mild cognitive impairment or in persons who are in the early stages of dementia: these studies too have found that the temporal GM is most severely affected, followed by several subregions in the frontal and parietal lobes.[51-54] This pattern of lobar atrophy has also consistently been confirmed by various post-mortem studies in dementia patients.[17, 18] Moreover, we found this same pattern of lobar GM atrophy even after exclusion of persons with objective or subjective cognitive deficits at baseline. Although we do not separately diagnose mild cognitive impairment in our cohort, persons with subjective memory complaints AND z-memory below 1.5 SD of age- and education-specific means closely fit the criteria.[55] Our findings therefore emphasize that dementia has a long pre-clinical phase in which atrophic changes are already taking place without these being clinically apparent, even as mild cognitive impairment.[8] More importantly, this indicates that the actual moment of clinical diagnosis of dementia and mild cognitive impairment is rather arbitrary and does not accurately reflect the actual disease process, which may have started several years before. Finally, we found that after excluding persons with subjective memory complaints

OR z-memory below 1.5 SD brain atrophy measures were no longer predictive of dementia. Therefore, future studies should also focus on brain changes other than atrophy taking place pre-clinically in those persons, who are cognitively normal at baseline, but do develop dementia during follow-up.

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Chapter 3.2

Vascular Brain Disease in relation to Depression in the Elderly

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ABSTRACT

Background: Whereas cross-sectional studies show an association between vascular brain disease and depression, longitudinal data are scarce. In a population-based study we investigated this relationship both cross-sectionally and longitudinally.

Methods: 479 persons (60-90 years) underwent brain MRI. Brain atrophy, white matter lesions (WML) and brain infarcts reflected vascular brain disease. At baseline (1995-1996) and follow-up examinations we identified persons with depressive symptoms and syndromes using CES-D and psychiatric interview. Moreover, medical records were continuously monitored to identify incident depression. Follow-up was complete until October 2005.

Results: At baseline 36 persons had depressive symptoms. Brain atrophy, WML, and infarcts were associated with presence of depressive symptoms. During follow-up 92 persons developed depressive symptoms, of whom 35 depressive syndrome. There was no association of any MRI-marker with incident depressive symptoms or syndromes.

Conclusions: Markers of vascular brain disease were associated with depression cross-sectionally. However, no relationship was present between these markers and risk of depression longitudinally.

INTRODUCTION

Accumulating evidence indicates that vascular disease and depression in the elderly are closely related.[1, 2] This has led to the 'vascular depression' hypothesis,[3] which postulates a vascular basis of late-life depression.[4-6] Magnetic resonance imaging (MRI) can be used to visualize markers of vascular brain disease, including white matter lesions (WML), brain infarcts, and brain atrophy. Various cross-sectional studies have shown an association of several MRI-markers with depression.[4, 7-10] However, cross-sectional studies do not allow interpretation of the results with respect to cause and effect. Few studies have investigated the relationship of WML with depression longitudinally, but found inconsistent results.[9, 11-13] No study investigated brain atrophy and only one also considered brain infarcts.[9]

We investigated the relationship of several MRI-markers of subclinical vascular brain disease both cross-sectionally with prevalent depression, and longitudinally with incident depression.

METHODS AND MATERIALS

Study population

This study is based on an age-stratified (60-90 years) random sample of 563 participants from the population-based Rotterdam Study,[14] who underwent brain MR-imaging in 1995-1996 as part of the Rotterdam Scan Study.[15] The institutional medical ethics committee approved the study and all participants gave written informed consent. All participants underwent a multi-sequence MRI examination. Of the 563 participants, 73 did not complete MRI examination due to claustrophobia or technical reasons, and 4 persons did not undergo psychiatric assessment at baseline. Furthermore, persons using anti-depressants at baseline but without depressive symptoms (n=7) were excluded, because we could not determine whether the indication for using these drugs (namely depression) was still present. Therefore, a total of 479 persons were available for the present analysis.

MRI measures at baseline

Image acquisition, classification algorithm, and validation steps have been described elsewhere.[15] In summary, we used the k-nearest-neighbor (kNN) classifier to classify voxels into cerebrospinal fluid, grey matter, normal white matter, and WML. Using non-rigid transformation, non-cerebral tissues (e.g. eyes, skull) were stripped. For measurement of lobar and deep central brain volumes, we first created an atlas, in which the lobes were labeled according to a slightly modified version of the segmentation protocol as described by Bokde et al.[16] Subsequently, we used validated non-rigid transformation to transform this atlas to each brain. Brain infarcts were rated visually as focal hyperintensities on T2-weighted images, 3 mm in size or larger and with a corresponding prominent hypointensity on T1-weighted images.[15]

Assessment of depression

The assessment of depression has been extensively described elsewhere.[17] In short, at the baseline visit and during three follow-up rounds (1997-1999, 1999-2000, 2000-2001) the Center for Epidemiological Studies Depression Scale (CES-D) was used as screening tool with a cut-off of 16. Screen-positive individuals then underwent the Present State Examination[18] to diagnose major depression, dysthymia, and minor depression. Moreover, medical and pharmacy records of participants (e.g. hospital discharge letter, specialists' reports, and notes of general practitioners) were continuously monitored for depressive episodes and for start of anti-depressants during the entire follow-up period by automated linkage of the general practitioners' and pharmacists' databases with the study database.

Depressive episodes were classified as depressive symptoms if persons were CES-D positive; or had at least one core symptom of depression (feeling depressed or loss-of-interest) recorded in medical files; or started anti-depressants (without documentation of clinical

symptoms). Depressive symptoms were further classified as depressive syndrome if persons were diagnosed as suffering from major depression, minor depression, or dysthymia according to the psychiatric interview or medical files. Follow-up for incident depressive symptoms and syndromes was complete until October 1st 2005.

Covariates

Participants underwent the Mini-Mental State Examination, and they were asked about their education level. Other covariates – as previously described[15] – included smoking, blood pressure, diabetes mellitus, body-mass index, and intima-media thickness.

Statistical analysis

All brain measure volumes were expressed as percentage of intra-cranial volume. Total white matter was the sum of normal white matter and WML. WML were analyzed as ln(WML volume), because of skewness of the untransformed measures.

We used logistic regression to calculate odds ratios for presence of depressive symptoms associated with brain imaging markers.

For the longitudinal analyses, we first excluded persons with depressive symptoms at baseline (n=36). We used Cox' proportional-hazards models to calculate hazard ratios for incident depressive symptoms or syndromes associated with brain imaging markers. Persons were followed-up until onset of depressive symptoms or depressive syndrome, loss to follow-up, or October 1st 2005, whichever came first.

RESULTS

Table 1 shows the baseline characteristics of the study population. At baseline, 36 persons had depressive symptoms, of whom 6 had a depressive syndrome. The smaller the brain volume the more likely persons were to have depressive symptoms (Table 2). At the lobar level, particularly parietal and temporal lobe atrophy were associated with depressive symptoms. The likelihood of having depressive symptoms increased with increasing volume of WML, especially in the frontal lobe and deep central region, and with presence of brain infarcts. Numbers were too small to perform separate analyses for prevalent depressive syndromes (n=6).

During 3,373 person-years of follow-up (mean 7.5 years) a total of 92 persons developed depressive symptoms, of whom 35 suffered from a depressive syndrome. Global nor lobar brain tissue volumes were associated with depressive symptoms or depressive syndromes (Table 3). Neither WML nor brain infarcts were associated with incident depressive symptoms or depressive syndromes. Additional adjustment for cardiovascular risk factors did not change the results.

Table 1. Baseline characteristics of the study population.		
N		**479**
Age, yr		73.4 (7.8)
Women		50%
Primary education only		30%
MMSE, score		27.7 (2.1)
Current smokers		18%
Former smokers		54%
Systolic blood pressure, mmHg		146 (21)
Diastolic blood pressure, mmHg		77 (12)
Diabetes mellitus		5.0%
Body mass index, kg/m^2		26.2 (3.5)
Intima media thickness, mm		0.87 (0.14)
Whole brain volume, %ICV		77.4 (3.6)
Frontal lobe volume, %ICV		27.4 (1.9)
	Parietal lobe volume, %ICV	15.8 (1.1)
	Occipital lobe volume, %ICV	9.0 (0.7)
	Temporal lobe volume, %ICV	15.5 (0.9)
	Deep central region, %ICV*	9.7 (0.5)
Grey matter, %ICV		46.6 (4.1)
Normal white matter, %ICV		29.5 (6.3)
Total white matter, %ICV		30.8 (5.7)
White matter lesions, %ICV		1.33 (1.51)
Brain infarcts		28%

Values are percentages or means (standard deviation)
MMSE: Mini-Mental State Examination
ICV: Intra-cranial volume
*The deep central region includes the corpus callosum, insular cortex, basal ganglia, and the white matter surrounding the basal ganglia

DISCUSSION

In this population-based cohort study of elderly persons we found that structural markers of vascular brain disease were cross-sectionally related to presence of depressive symptoms. However, we did not find any association between structural brain markers and incident depressive symptoms or depressive syndromes.

Strengths of our study include the population-based setting, and the cross-sectional as well as longitudinal design of the study with more than seven years of follow-up. Due to close collaboration with general practitioners and other health care institutions, the follow-up for depressive episodes was virtually complete. Moreover, contrary to other studies we investigated various markers of vascular brain disease using automated quantification techniques.A possible limitation is that we did not have reliable data on history of depression at baseline.Therefore, it is possible that some persons who developed depression or remained

Table 2. Cross-sectional association between brain tissue volumes and prevalent depressive symptoms.

	Odds ratio (95% confidence interval) for presence of depressive symptoms (n=36), including depressive syndromes (n=6)	
Brain tissue volumes*	Adjusted for age, sex, education, and MMSE-score	Additionally adjusted for cardiovascular risk factors§
Global brain tissue volumes		
Whole brain volume	0.52 (0.31-0.87)	0.58 (0.34-0.99)
Grey matter	0.95 (0.67-1.33)	0.95 (0.67-1.34)
Normal white matter	0.70 (0.45-1.08)	0.73 (0.47-1.13)
Total white matter	0.81 (0.53-1.22)	0.86 (0.57-1.29)
Lobar brain tissue volumes†		
Frontal lobe	0.68 (0.43-1.07)	0.75 (0.48-1.19)
Parietal lobe	0.56 (0.35-0.91)	0.61 (0.37-0.99)
Occipital lobe	0.87 (0.60-1.27)	0.89 (0.61-1.31)
Temporal lobe	0.65 (0.43-0.99)	0.65 (0.42-1.00)
Deep central region	1.08 (0.76-1.54)	1.17 (0.81-1.69)
WML volume‡ and brain infarcts		
Global WML	1.55 (0.98-2.46)	1.71 (1.03-2.82)
Frontal WML	1.68 (1.07-2.63)	1.85 (1.14-3.00)
Parietal WML	1.30 (0.85-1.98)	1.39 (0.87-2.21)
Occipital WML	1.44 (0.93-2.23)	1.61 (0.99-2.63)
Temporal WML	1.35 (0.88-2.08)	1.41 (0.89-2.25)
Deep WML	1.82 (1.13-2.96)	1.99 (1.19-3.35)
Brain infarcts (yes versus no)	2.34 (1.12-4.89)	2.54 (1.19-5.42)

MMSE Mini-Mental State Examination, WML white matter lesion

* expressed per standard deviation increase

† these volumes included grey matter and total white matter together

‡ all white matter lesion volumes were natural log transformed

§ smoking, systolic blood pressure, diastolic blood pressure, diabetes mellitus, body-mass index, intima media thickness

free of depression during follow-up might actually already have had a depression before baseline. This misclassification would lead to an underestimation of the true effect. A final consideration is that we excluded persons who used anti-depressants at baseline. However, post hoc analyses including these persons in either the depressed or non-depressed group yielded unchanged results.

In our cross-sectional analyses we found brain atrophy, brain infarcts and WML to be related to depressive symptoms (including depressive syndromes). This fits well with various previous studies.[4, 8, 9] Furthermore, when investigating at a lobar level our results are also in accordance with published data showing that particularly atrophy in the parietal and frontal lobes, and frontal and deep WML are related to depression.[4, 10, 19]

However, in our longitudinal analysis we did not find an association between any MRI-marker and incident depressive episodes. This is in contrast with results from the 3C-Dijon Study and LADIS Study,[12, 13] but in line with data from the PROSPER Study and the Cardiovascular Health Study.[9, 11] A possible explanation for the discrepancies might be

Table 3. Risk of depressive symptoms and syndromes associated with brain tissue volumes.

Brain tissue volumes*	Hazard ratio (95% CI) for incident depressive symptoms (including depressive syndromes) (N=92)	Hazard ratio (95% CI) for incident depressive syndromes (N=35)
Global brain tissue volumes		
Whole brain volume	1.04 (0.78-1.41)	0.83 (0.51-1.34)
Grey matter	0.92 (0.74-1.14)	0.96 (0.67-1.36)
Normal white matter	1.10 (0.85-1.41)	0.90 (0.59-1.38)
Total white matter	1.10 (0.87-1.40)	0.96 (0.64-1.43)
Lobar brain tissue volumes†		
Frontal lobe	0.88 (0.68-1.15)	0.87 (0.57-1.33)
Parietal lobe	1.04 (0.81-1.33)	0.85 (0.57-1.27)
Occipital lobe	1.08 (0.87-1.34)	1.09 (0.78-1.53)
Temporal lobe	1.14 (0.90-1.45)	0.85 (0.58-1.26)
Deep central region	1.10 (0.88-1.38)	1.00 (0.69-1.45)
WML volume‡ and brain infarcts		
Global WML	0.89 (0.71-1.11)	0.85 (0.60-1.21)
Frontal WML	0.88 (0.71-1.09)	0.86 (0.61-1.20)
Parietal WML	0.88 (0.72-1.08)	0.89 (0.64-1.22)
Occipital WML	0.88 (0.71-1.09)	0.89 (0.63-1.25)
Temporal WML	0.93 (0.75-1.15)	0.89 (0.63-1.25)
Deep WML	0.93 (0.74-1.17)	0.91 (0.63-1.32)
Brain infarcts (yes versus no)	0.87 (0.52-1.46)	0.93 (0.38-2.25)

Values are adjusted for age, sex, education and Mini-Mental State Examination-score. Persons were censored at onset of depressive syndrome, onset of depressive symptoms, date last known to be alive in case of loss to follow-up, or October 1st 2005, whichever came first.
CI confidence interval, WML white matter lesion
* expressed per standard deviation increase
† these volumes included grey matter and total white matter together
‡ all white matter lesion volumes were natural log transformed

differences in assessment of depression, which ranged from using only a single test[9, 13] to various combinations of different psychiatric tests.[11, 12] Furthermore, differences in source population – clinical trial,[11] a clinical setting,[13] or a population-based setting[9, 12] – might also have contributed to inconsistent findings between studies.

The question remains what underlies the strong cross-sectional association of brain markers with depression, if not a causal relationship from vascular brain disease to depression. One explanation might be that the causal pathway works the other way around, i.e. depression causes vascular brain disease. We did not have volumetric data on progression of white matter lesions in the Rotterdam Scan Study to investigate this issue. However, two studies have indeed reported a larger increase in WML in depressed compared with non-depressed persons.[9, 12] Though it is unclear how depression could lead to vascular brain

disease and brain atrophy, possible mechanisms include platelet dysfunction, hypotensive episodes, unhealthy lifestyle choices (e.g. smoking, drinking), and elevated cortisol levels in the brain, which in turn can cause glucocorticoid-mediated neurotoxicity.[1, 5, 20] Another possible explanation is a common etiology (e.g. genetic predisposition) linking depression with vascular brain disease cross-sectionally, but not necessarily longitudinally. Indeed, in a twin study Scherrer et al. showed that the co-occurrence of cardiovascular disease and depression is partly explained by common genetic risk factors.[21] Finally, it is possible that vascular brain disease does not cause depression, but is related to persistence of depression.[9] Still, additional longitudinal studies are needed to test these hypotheses and further disentangle the exact mechanisms linking vascular brain disease and depression.

In conclusion, we found that MRI-markers of vascular brain disease were strongly associated with depression cross-sectionally. However, our study emphasizes that repeatedly finding an association cross-sectionally does not necessarily demonstrate causation, as we did not find any evidence for the 'vascular depression' hypothesis relating these brain markers to incident depression longitudinally.

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Chapter 3.3

Brain Tissue Volumes and Small Vessel Disease in relation to the Risk of Mortality

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ABSTRACT

Brain atrophy and small vessel disease increase the risk of dementia and stroke. In a population-based cohort study (n=490; 60-90 years) we investigated how volumetric measures of atrophy and small vessel disease were related to mortality and whether this was independent of incident dementia or stroke. Brain volume and hippocampal volume were considered as measures of atrophy, whereas white matter lesions (WML) and lacunar infarcts reflected small vessel disease. We first investigated all-cause mortality in the whole cohort. In subsequent analyses we censored persons at incident dementia or incident stroke. Finally, we separately investigated cardiovascular mortality. The average follow-up was 8.4 years, during which 191 persons died. Brain atrophy and hippocampal atrophy, as well as WML increased the risk of death. The risks associated with hippocampal atrophy attenuated when censoring persons at incident dementia, but not at incident stroke. Censoring at either incident dementia or stroke did not change the risk associated with brain atrophy and WML. Moreover, WML were particularly associated with cardiovascular mortality.

INTRODUCTION

Subclinical structural brain changes, which can be visualized on magnetic resonance imaging (MRI),[1,2] are important indicators of future adverse neurological events. Atrophy of the brain and the hippocampus are strong predictors of dementia and cognitive decline,[3-5] and presence of cerebral small vessel disease (white matter lesions (WML) and lacunar infarcts) too is related to incident dementia[6] and stroke.[7] A few clinical studies have reported that markers of cerebral small vessel disease are also associated with increased mortality.[8,9] The population-based Cardiovascular Health Study reported that WML and ventricular size, which is an indirect measure of brain atrophy, were related to mortality independent of incident dementia.[10] The MRI measures used in these studies were rated visually on a semi-quantitative scale.

The question remains how volumetric and therefore direct measures of atrophy and small vessel disease relate to mortality, and whether the relationship of these volumetric MRI measures with mortality is independent of adverse neurological events, such as dementia or stroke.

In the population-based Rotterdam Scan Study we investigated how volumetric measures of brain atrophy, hippocampal atrophy and markers of cerebral small vessel disease are related to all-cause and cardiovascular mortality. Furthermore, we investigated whether the relationships were independent of incident dementia or stroke.

METHODS

Participants

The Rotterdam Scan Study is a large population-based cohort study in the Netherlands investigating age-related brain changes on MRI.[7] The medical ethics committee of the Erasmus Medical Center, Rotterdam, The Netherlands, approved the study. At baseline (1995-1996), we randomly invited participants, aged 60-90 years, stratified by sex and 5-year age strata from the population-based Zoetermeer Study[11] and Rotterdam Study[12] to participate in the Rotterdam Scan Study. After exclusion of individuals who were demented or had MRI contraindications, 1,077 participants gave their written informed consent to participate in the study. At baseline, participants underwent an interview at home and a physical examination at the research center. Furthermore, an MRI scan of the brain was performed. The present study is restricted to persons originating from the Rotterdam Study (n=563), because they underwent an additional high-resolution 3D MR sequence. Among these 563 participants, 52 developed claustrophobia during MRI acquisition, which prohibited completion of the scanning protocol. Twenty-one datasets were unusable for volumetric analysis due to excessive ghosting artifacts (n=5), scanning outside the range of coil sensitivity (n=10), or other reasons (n=6), leaving a total of 490 participants in our present study.

MRI measures at baseline

MR brain imaging was performed on a 1.5-Tesla MRI System (VISION MR, Siemens AG, Erlangen, Germany). The protocol included three axial scans, i.e. T1-weighted (repetition time (TR)=700, echo time (TE)=14, number of excitations (NEX)=1, matrix 192x256, field of view (FOV)=256x256, flip angle=80°, 20 slices), proton-density (PD) weighted (TR=2200, TE=20, NEX=1, matrix=192x256, FOV=256x256, flip angle=80°, 20 slices) and T2-weighted (TR=2200, TE=80, NEX=1, matrix=192x256, FOV=256x256, flip angle=80°, 20 slices). Slice thickness was 5 mm with an interslice gap of 20%. Furthermore, a high-resolution, inversion-recovery double contrast, 3-D half-Fourier acquisition single-shot turbo spin echo (HASTE) sequence was included (inversion time=4400ms, TR=2800, matrix=192x256, FOV=256x256, 128 contiguous sagittal slices of 1.25 mm). The interpolated voxel dimensions were 1x1x1.25mm^3. Two HASTE modules were sequentially acquired after the inversion pulse (effective TE of 29 ms and 440 ms). Each HASTE module combined nonselective radio frequency excitations to provide a short interecho spacing of 3.9 ms. We used the proton-density, T2-weighted and the first HASTE module (HASTE-Odd) for our multi-spectral volumetry.

Data were stored onto a Linux Workstation. Preprocessing steps and the classification algorithm have been described.[13, 14] In summary, preprocessing included co-registration, non-uniformity correction and variance scaling. Afterwards, we used the k-nearest-neighbor classifier to classify scans into cerebrospinal fluid (CSF), grey matter (GM), normal white

matter (WM), and WML.[15] In order to minimize any misclassification of partial volume voxels as WML around cortical GM, we registered a manually created mask, within which voxels could be classified as WML. To remove non-brain tissue and infratentorial tissue, we used non-rigid transformation[16, 17] to register to each brain a template scan, in which all these tissues were manually masked. For an example of the classification result, see Ikram et al.[14]

Finally, all scans were verified for proper classification by two trained neuro-imagers, blinded for clinical information. If needed, any voxels that were misclassified were manually reclassified appropriately. This was needed in only 45 of the scans, mostly due to misclassification because of slight motion artifacts. Validation methods and results have been described.[13, 14] We used similarity indices (SI)[18] and intra-class correlation coefficients (ICC) as validation measures. The SI between the automated classification and manual labeling was 0.91 for CSF, 0.92 for GM, 0.93 for normal WM and 0.63 for WML. The ICC between the automated classification and manual labeling was 0.89 for CSF, 0.94 for GM, 0.80 for normal WM and 0.84 for WML. The SIs and ICCs between two neuro-imagers were only slightly higher. These numbers indicate very good to excellent agreement and overlap.[18]

Hippocampal volumes were manually outlined on coronal HASTE-Odd scans reconstructed perpendicular to the long axis of the hippocampus.[19] Volumes were calculated by summing the areas multiplied by slice thickness in both the left and right hemispheres. Lacunar infarcts were rated visually as focal hyperintensities on T2-weighted images, 3 mm in size or larger and with a corresponding prominent hypointensity on T1-weighted images. Intra-rater agreement for detection of infarcts was good (κ=0.80).[7]

Follow-up for mortality

Information on vital status was obtained from the municipal health authorities in Rotterdam on a biweekly basis. Also, the general practitioners in the study area reported deaths on a continuous basis. After notification, information on cause and circumstances of death was obtained from general practitioners and by checking the medical records. Two research physicians independent from each other and blinded for brain tissue volumes coded all reported events according to the International Classification of Diseases, 10th edition (ICD-10).[20] If the cause of death was coded as I20-I25, I46, I50, I60-70 and R96, the cause of death was labeled as death from a cardiovascular cause. Codes on which the research physicians disagreed were discussed in order to reach consensus. Finally, a medical expert in cardiovascular disease, whose judgment was considered final, reviewed all cardiovascular events.

Participants were followed from date of entry until date of death, date at which they were lost to follow-up, or August 15th 2006, whichever came first. Follow-up data on vital status were nearly complete (98.4%).

Follow-up and diagnosis of dementia and stroke

After entrance into the Rotterdam Scan Study, participants were continuously monitored for major events, including stroke and dementia, through automated linkage of the study database with files from general practitioners, the municipality and the Regional Institute for Outpatient Mental Health Care. Also, nursery home physicians' files and files from general practitioners of participants who moved out of the district were reviewed.

In addition, during two follow-up visits to the research center (1999-2000, 2001-2002) all participants were screened for dementia following a three-step protocol as used in the Rotterdam Study.[6, 21] Briefly, two brief tests of cognition (Mini-Mental State Examination (MMSE)[22] and Geriatric Mental State schedule (GMS)[23] organic level) were used to screen all subjects. Screen-positives (MMSE score < 26 or GMS organic level > 0) underwent the Cambridge examination for mental disorders of the elderly (Camdex).[24] If needed for diagnosis, a neuropsychologist further examined persons suspected of having dementia. The diagnosis of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III-R) by a panel consisting of a research physician, a neurologist and a neuropsychologist.

Stroke was defined as rapidly developing clinical signs of focal disturbance of cerebral function with no apparent cause other than a vascular origin, with duration of more than 24 hours. For reported events, additional information 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 diagnoses.

Assessment of confounders

At baseline, participants answered a computerized questionnaire at home and underwent physical examination at the research center to obtain information on medical history and current health status. Smoking status was verified and participants were classified into one of three categories: current smoker, former smoker, or never smoker. At the research center, blood pressure in a sitting position was measured twice on the right upper arm using a random-zero sphygmomanometer. In the analyses the average of these two measurements was used. Diabetes mellitus was defined as random or post-load serum glucose level exceeding 11.1 mmol/l or the use of oral blood glucose lowering drugs or insulin. We calculated body-mass index as weight/height^2 (kg/m^2). Participants underwent B-mode ultrasonography of both carotid arteries to measure intima-media thickness.

In the analyses, we used baseline values (1995-1996) of these potential confounders.

Data analysis

All volumes were expressed as percentage of intra-cranial volume (= CSF + GM + normal WM + WML) to correct for individual head-size differences. Therefore, a larger relative volume of CSF indicates a smaller whole brain volume. Total WM was defined as the sum

of normal WM and WML. WML volume was further natural log transformed because of skewness of the untransformed measure.

With Cox' proportional hazards model we calculated hazard ratios (HR) for all-cause mortality associated with brain tissue volumes, WML volume, and brain infarcts (yes versus no). We first investigated brain tissue volumes and WML volume in quartiles of their distribution and calculated trends over the quartiles. Afterwards, we used these volumes as continuous measures (per standard deviation (SD)). We also combined quartiles of CSF volumes and WML volumes; and calculated interaction terms between the continuous measures of CSF and WML.

To investigate the effect of incident dementia or stroke on these associations, we subsequently performed analyses after censoring persons at onset of dementia, or at occurrence of stroke. For better comparison with the Cardiovascular Health Study,[10] we also performed these analyses by taking incident dementia or stroke as time-dependent covariate. Finally, we conducted separate analyses for mortality due to a cardiovascular cause.

All analyses were adjusted for age, sex (model I), and additionally for systolic blood pressure, diastolic blood pressure, body-mass index, current smoking, former smoking, intima-media thickness and diabetes mellitus (model II). Results are presented with corresponding 95% confidence interval (CI). Analyses were done using SPSS 11.0.1, Illinois, USA for Windows.

RESULTS

Table 1 shows the baseline characteristics of the study population. Follow-up was complete until August 15th 2006. During a mean follow-up of 8.4 years a total of 191 persons died (incidence rate 46.3 deaths per 1,000 person-years), of whom 49 due to a cardiovascular cause. A total of 46 persons developed dementia, of whom 8 died within one year after onset and a total of 32 died during the whole of the follow-up period (6 of these 32 died due to a cardiovascular cause). Forty-eight persons suffered a stroke, of whom 18 died within one year after stroke and 37 died during the whole of the follow-up period (19 of these 37 died due to a cardiovascular cause).

Table 2 shows the risk of all-cause mortality after categorizing brain tissue volumes into quartiles. Persons in the upper quartile of CSF volume had an increased risk of death compared to persons in the lowest quartile. Persons with larger GM or larger total WM had a decreased risk of death, though this did not reach statistical significance. Those with a larger hippocampal volume also had a decreased risk of all-cause mortality compared to the lowest quartile. Larger WML volume was associated with an increased risk of death. When analyzing brain tissue volumes continuously, similar associations were found (Table 2). Persons in the highest quartile of both CSF and WML volume had a more than three-fold

Table 1. Baseline characteristics of the study population.	
	Total cohort, n=490
Age, yr	73.4 (7.9)
Women, n	249 (51)
Systolic blood pressure, mmHg	146 (21)
Diastolic blood pressure, mmHg	77 (12)
Body mass index, kg/m^2	26.2 (3.5)
Intima-media thickness, mm	0.87 (0.14)
Diabetes mellitus, n	24 (5)
Current smoker, n	87 (18)
Former smoker, n	264 (54)
Cerebrospinal fluid, % of ICV	22.6 (3.7)
Grey matter, % of ICV	46.6 (4.1)
Normal white matter, % of ICV	29.5 (6.4)
Total white matter, % of ICV	30.8 (5.7)
Hippocampus, % of ICV	0.57 (0.08)
White matter lesions, % of ICV [a]	-0.33 (1.26)
Lacunar infarcts, n	112 (23)

Values are means (standard deviation) for continuous variables, or numbers (percentage) for dichotomous variables. ICV intracranial volume

[a] natural log transformed

increased risk of death as compared with those in the lowest quartile of both CSF and WML volume (hazard ratio 3.06, 95% CI 1.34-6.99); the interaction term, however, was not significant (p=0.30), indicating an additive rather than a multiplicative effect.

Adjusting for cardiovascular risk factors only marginally changed the results (data not shown).

After censoring persons at onset of dementia, hippocampal volume was no longer associated with all-cause mortality (Table 2). The risk associated with CSF volume, WML volume and lacunar infarcts remained largely unchanged. When censoring persons at occurrence of stroke, the associations did not change (Table 2). Adding incident dementia or stroke as time-dependent covariate to the model yielded results similar to censoring persons at incident dementia or stroke.

Finally, hippocampal volume and CSF volume were not associated with cardiovascular mortality (Table 3). In contrast, WML infarcts showed a strongly increased risk of cardiovascular death, whereas the risk associated with lacunar infarcts also increased, though it did not reach statistical significance (Table 3, model I). Additional adjustment for cardiovascular risk factors did not change the associations (Table 3, model II), nor did excluding fatal strokes from the cardiovascular deaths (data not shown).

Table 2. Risk of all-cause mortality associated with brain tissue volumes and markers of small vessel disease.

	Cerebrospinal fluid	Grey matter	Normal white matter	Total white matter	Hippocampus	White matter lesions [a]	Lacunar infarct
1st quartile	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	NA
2nd quartile	1.19 (0.67-2.11)	0.79 (0.53-1.18)	0.72 (0.50-1.06)	0.94 (0.65-1.36)	0.65 (0.44-0.95)	1.10 (0.69-1.77)	
3rd quartile	1.49 (0.84-2.67)	0.93 (0.62-1.39)	0.73 (0.49-1.10)	0.83 (0.55-1.26)	0.66 (0.43-0.99)	1.30 (0.82-2.05)	
4th quartile	1.97 (1.07-3.63)	0.89 (0.59-1.34)	0.65 (0.41-1.04)	0.81 (0.52-1.28)	0.68 (0.43-1.08)	2.05 (1.32-3.20)	
p-trend	0.01	0.76	0.06	0.30	0.06	<0.001	NA
Per SD	1.39 (1.13-1.71)	0.95 (0.82-1.10)	0.83 (0.70-0.99)	0.91 (0.78-1.08)	0.85 (0.73-1.01)	1.38 (1.16-1.65)	1.25 (0.90-1.73) [b]
Per SD, participants censored at onset of dementia	1.27 (1.02-1.60)	1.01 (0.86-1.19)	0.84 (0.70-1.01)	0.90 (0.75-1.08)	0.94 (0.79-1.13)	1.31 (1.08-1.58)	1.34 (0.94-1.90) [b]
Per SD, participants censored at occurrence of stroke	1.48 (1.18-1.86)	0.93 (0.79-1.09)	0.85 (0.69-1.02)	0.91 (0.76-1.09)	0.77 (0.64-0.93)	1.30 (1.07-1.58)	1.22 (0.84-1.75) [b]

Values are hazard ratios, adjusted for age and sex, with 95% confidence interval. Volumetric measures are expressed as percentage of intra-cranial volume. SD standard deviation, NA not applicable

[a] natural log transformed

[b] yes versus no

DISCUSSION

In this population-based cohort study of elderly, we found that brain atrophy, hippocampal atrophy and WML increased the risk of all-cause mortality. The risk of mortality associated with hippocampal atrophy diminished after censoring persons at onset of dementia, but not after censoring at occurrence of stroke. The risk of mortality associated with brain atrophy and WML remained unchanged after censoring for either incident dementia or stroke. Finally, WML were particularly related to cardiovascular mortality, whereas brain and hippocampal atrophy were not.

Strengths of our study include the population-based setting, large sample size, meticulous case finding, and virtually complete follow-up for mortality and dementia and stroke. Most importantly, in contrast to previously used semi-quantitative indirect rating scales,[10, 25] we used automated quantification of brain MR-images to obtain volumetric and direct measures of brain tissue volumes. A limitation of our study could be that in some cases the cause of death could have been misclassified. However, because research physicians were blinded for brain tissue volumes, any misclassification is likely to have been non-differential and would lead to an underestimation of the effect. Another consideration is that despite our meticulous efforts to detect all

Table 3. Risk of cardiovascular mortality associated with brain tissue volumes and markers of small vessel disease.

Brain tissue	Risk of cardiovascular mortality	
	Model I	Model II
Cerebrospinal fluid, % of ICV (per SD)	1.35 (0.89-2.02)	1.38 (0.91-2.09)
Grey matter, % of ICV (per SD)	0.91 (0.69-1.21)	0.98 (0.73-1.31)
Normal white matter, % of ICV (per SD)	0.81 (0.58-1.13)	0.74 (0.53-1.03)
Total white matter, % of ICV (per SD)	0.97 (0.70-1.33)	0.90 (0.66-1.24)
Hippocampus, % of ICV (per SD)	0.97 (0.70-1.35)	0.96 (0.68-1.36)
White matter lesions, % of ICV (per SD) [a]	2.01 (1.37-2.96)	2.52 (1.65-3.84)
Lacunar infarct (yes versus no)	1.70 (0.90-3.21)	1.88 (0.98-3.60)

Values are hazard ratios with 95% confidence interval. ICV intra-cranial volume, SD standard deviation
Model I: adjusted for age, sex
Model II: adjusted for age, sex, systolic blood pressure, diastolic blood pressure, body-mass index, current smoking, former smoking, intima-media thickness and diabetes mellitus
[a] natural log transformed

dementia cases, very early and mild cases of dementia might have been missed by our follow-up screening exams. Especially, upon death or towards the end of the study period these persons would also still have been too mildly demented to be picked up by our continuous monitoring. This would have led to a slight underestimation of the total number of dementia cases in our study.

We found that brain atrophy and hippocampal atrophy indicated an increased risk of all-cause mortality, which is in line with results from the Cardiovascular Health Study.[10] Both brain atrophy and hippocampal atrophy are also known predictors of cognitive decline and dementia.[3, 6, 19] The risk of death associated with hippocampal atrophy attenuated after censoring for dementia. This suggests that it is the dementia process itself, of which hippocampal atrophy can be a marker, rather than a small hippocampus itself, that is associated with increased mortality.[26-28] However, the causes of increased mortality in dementia patients are still largely unknown.[28]

The attenuation of the risk of mortality after taking into account incident dementia was only present for hippocampal atrophy and not for brain atrophy. This is in accordance with previous findings that in the general population hippocampal atrophy is more strongly associated with dementia than is brain atrophy.[29]

We found that WML increased the risk of all-cause mortality, which fits with observations from several other studies in demented or depressed patients,[8, 9] or in the general population.[10, 25] Although the presence of cerebral small vessel disease is associated with cognitive decline,[4] dementia,[6] and stroke,[7] censoring persons at onset of dementia or occurrence of stroke only marginally changed the risk estimates for all-cause mortality associated with WML and lacunar infarcts. Moreover, in our dataset WML and to a lesser extent lacunar infarcts also indicated a strongly increased risk of cardiovascular death, even after excluding fatal strokes. This suggests that persons with cerebral small vessel disease not only have an increased risk of adverse neurological outcomes, but are also prone to other cardiovascular

events, which can lead to their death. However, because cerebral small vessel disease is closely associated with vascular risk factors,[30, 31] confounding may play a role in the association between cerebral small vessel disease and cardiovascular mortality. We found that additional adjustment for vascular risk factors did not change this association (model II). Thus, presence of cerebral small vessel disease may reflect cardiovascular damage in the whole body better than these cardiovascular risk factors. An additional explanation could be that cerebral small vessel disease also reflects other cardiovascular determinants that we did not measure in our study, e.g. genetic factors. Finally, the possibility of residual confounding due to measurement error cannot be fully ruled out.

Our automated classification algorithm yielded total volume of WML and did not distinguish WML according to their location. Given the proposed difference between subcortical and periventricular WML in etiology and cognitive outcomes,[32-34] future studies should investigate whether the prognosis for death also differs according to location of WML.

In conclusion, we showed that brain atrophy and hippocampal atrophy are related to death probably by leading to dementia. In contrast, presence of cerebral small vessel disease indicates an increased risk of death, in particular cardiovascular death, independent of incident dementia or stroke. This emphasizes the need of studies investigating whether installment of preventive strategies in those with atrophy or cerebral small vessel disease is indicated and effective in preventing mortality.

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Part 4

Chapter 4.1

Genome-wide Association Studies of Incident Total Stroke and Ischemic Stroke: Meta-analysis and Replication from the CHARGE Consortium

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ABSTRACT

Background: To identify genetic variants underlying stroke, we performed a prospective meta-analysis of genome-wide association data in white subjects from four large cohort studies comprising the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium, and replicated our finding in an African-American cohort.

Methods: Each discovery cohort used genotype information to impute to HapMap's CEU panel and age- and sex-adjusted Cox models to relate 2.2 million SNPs to incident stroke. Study-specific findings were combined in a fixed-effects meta-analysis including 19,602 stroke-free individuals (mean age 63) who developed 1,544 initial incident strokes (1,164 ischemic) over an average follow-up of 11 years. Findings were replicated in 2,430 African-Americans (mean age 53), of whom 215 developed incident stroke (191 ischemic) over 15 years.

Results: Two intergenic SNPs at one locus were significantly associated with incident total and ischemic stroke ($p<5x10^{-8}$). For the first SNP, the risk per allele in the discovery cohort was 31% (95%CI:19-44%) higher for total stroke, and 39% (95%CI:27-46%) higher

for ischemic stroke yielding population attributable risks of 11 and 14%, respectively. In African-Americans, corresponding risks were 39% (95%CI:5-84%) and 43% (95%CI:6-92%). Conclusions: Our community-based GWAS uncovered a novel association for incident stroke. Exploring the epidemiological, molecular and clinical correlates of genetic variation at this locus may permit new insights into the pathophysiology of stroke.

INTRODUCTION

Identification of genetic and environmental risk factors for stroke is important because stroke is the leading neurological cause of mortality and morbidity.[1] Twin and familial-aggregation studies suggest that stroke risk has a substantial genetic component.[2-4] Whereas several monogenic disorders are known to cause stroke, the genes underlying stroke risk in the general population remain undetermined. Previous studies that used candidate gene as well as classical linkage approaches have yielded inconsistent findings.[5]

The application of unbiased genome-wide association study (GWAS) techniques to large samples has uncovered previously unsuspected common variants underlying the risk for other complex diseases such as diabetes[6] and coronary artery disease.[7, 8] A prior GWAS of stroke was limited by its small sample size (249 patients) and a case-control design restricted to prevalent cases.[9] Although an efficient and powerful design, case-control studies, especially for the study of late-onset disorders such as stroke, may suffer from selection and survival biases. Such biases are avoided in prospective studies of incident events but individual cohort studies have a limited number of events. We combined data from white participants in four large, prospective population-based cohort studies: the Atherosclerosis Risk in Communities (ARIC) Study,[10] the Cardiovascular Health Study (CHS),[11] the Framingham Heart Study (FHS),[12, 13] and the Rotterdam Study[14] to study the genetics of stroke. Together with the Aging Gene-Environment Susceptibility- Reykjavik Study (AGES-RS)[15] these studies form the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium. However, the AGES-RS does not have data on incident stroke and hence did not contribute to this meta-analysis. We present findings from a GWAS of incident total stroke and ischemic stroke as the primary and secondary outcomes, respectively, based on this discovery sample of four population-based cohorts with follow up of 19,602 white participants, and on a replication sample of 2,430 African-American participants in the ARIC study.

METHODS

Consortium organization

The CHARGE consortium includes large prospective community-based cohort studies that have genome-wide variation data coupled with extensive data on multiple phenotypes. All participating studies approved guidelines for collaboration, and a neurology working-group arrived at a consensus on phenotype harmonization, covariate selection and analytic plans for within-study analyses and meta-analysis of results. Each study has an Institutional Review Board that approved the consent procedures, examination and surveillance components, data security processes, genotyping protocols and current study design. All participants gave written informed consent for study participation and for use of DNA for genetic research.

Setting

Details of cohort selection, risk factor assessment and stroke surveillance in the four studies have been described previously.[10-14] The ARIC study enrolled 15,792 men and women (including 11,478 non-Hispanic whites) from four U.S. communities. Participants were between age 45 and 64 years at their baseline examination in 1987-1989. The CHS enrolled adults ≥65 years from four field centers (N=5,888 including 5,201 whites); the baseline examination was either in 1989-90 or 1992-93. The FHS is a single-site, study that comprises three generations of participants (N=10,333, virtually all white), the Original cohort followed since 1948,[12] their Offspring and spouses of the offspring followed since 1971,[13] and children from the largest offspring families followed since 2000 (Gen 3).[16] Gen 3 participants were not included in this analysis since they are young (mean age 40±9 years) and few have suffered strokes. The Rotterdam Study enrolled inhabitants from a district of Rotterdam (Ommoord) aged ≥55 years (N=7,983, virtually all white) at the baseline examination in 1990-93.

Study population

Participants who were stroke free entered the current study on the date of the blood draw used for their genotyping, and were then followed prospectively for incident stroke. FHS and Rotterdam participants were almost entirely European whites; so only non-Hispanic white ARIC and CHS participants were included in our analyses. Participants were excluded if they declined consent or failed genotyping. In addition, CHS did not genotype participants with any form of clinical cardiovascular disease at baseline. The meta-analysis included 7,686 participants from ARIC, 2,022 from CHS, 4,131 from FHS and 5,763 from the Rotterdam Study who met these criteria. Demographic and clinical characteristics of the study samples, assessed at the baseline examination, are shown in Table 1.

Table 1. Characteristics of Study Participants in Analysis of Incident Total stroke and Incident Ischemic Stroke				
STUDY	**ARIC**	**CHS**	**FHS**	**Rotterdam**
Number in sample*	7686	2022	4131	5763
Women %	53	55	55	59
Mean follow-up (yrs)	15	11	6	10
Mean Age (±SD)				
at DNA draw	54±6	73±6	66±12	69±9
at incident stroke	66±7	81±6	80±10	80±8
Number of incident total stroke	312	459	156	617
Number of incident ischemic stroke	277	389	131	367
Cardiovascular risk factor at baseline†				
Systolic Blood Pressure (mean ±SD)	118±17	138±22	131± 20	139±22
Diastolic Blood Pressure (mean ±SD)	72±10	71±11	74±10	74±12
Hypertension, %	27	61	52	61
Diabetes mellitus, %	6	14	12	10
Current Smoker,%	25	11	14	23
Prevalent CVD other than stroke, %	5	0‡	16	10

ARIC: Atherosclerosis Risk in Communities; CHS: Cardiovascular Health Study; FHS: Framingham Heart Study

*Only includes those genotyped persons who also provided consent for these analyses and had high-quality genotyping (met QC criteria).

† Definition of baseline characteristics was uniform across all four studies: Hypertension was defined using JNC-7 criteria as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg or being on antihypertensive treatment; Diabetes mellitus was defined as a casual or 2 hour post-prandial blood glucose ≥200mg/dl (11 mmol/L), a fasting blood glucose ≥126mg/dl (7 mmol/L), or use of insulin or oral hypoglycemic agents; CVD (cardiovascular disease) was defined as presence of congestive heart failure, coronary heart disease or intermittent claudication.

‡ In CHS, persons with prevalent CVD were not genotyped.

Stroke definition, surveillance and classification

All four studies defined stroke as a sudden onset focal neurological deficit of presumed vascular etiology lasting for at least 24 hours, or until death if the participant died less than 24 hours after onset of symptoms. Details of stroke surveillance and diagnostic criteria for stroke and stroke types in each of the four studies have been published.[17-20] Strokes were classified as ischemic, hemorrhagic or unknown type based on clinical and imaging criteria. For the analyses of total stroke, ischemic, hemorrhagic, and unknown strokes were included; subarachnoid hemorrhages were excluded.

Genotyping

The consortium was formed after the individual studies had finalized their GWAS platforms, and the four studies included used different platforms: the Affymetrix GeneChip® SNP Array 6.0 for ARIC, the Illumina HumanCNV370-Duo® for CHS, the Affymetrix GeneChip® Human Mapping 500K Array Set and 50K Human Gene Focused Panel® for FHS, and the Illumina Infinium HumanHap550-chip v3.0® for the Rotterdam Study. All studies used their genotype data to impute to the 2.5 million non-monomorphic, autosomal, SNPs described in HapMap (CEU population). Extensive quality control (QC) analyses have been performed in each cohort.

Statistical analyses within studies

Participants entered the analysis at the time of the DNA sample collection and were followed for their first stroke event; participants were censored at death or at the time of their last follow-up examination or health status update when they were known to be free of stroke. For the analyses of ischemic stroke, persons were also censored when they suffered an alternative type of stroke (hemorrhagic or unknown). Persons with a subarachnoid hemorrhage were censored at the time of the event (n=28). Each study fit an additive genetic model – a 1 degree of freedom trend test – relating genotype dosage (0 to 2 copies of the minor allele) to study trait. We used Cox proportional hazards models to calculate hazard ratios with corresponding 95% confidence intervals. Primary analyses were adjusted only for age and sex to avoid adjusting for covariates that might lie along a causal pathway. In addition, ARIC and CHS also adjusted for study site, and FHS adjusted for familial structure (by employing a Cox model with robust variance estimator clustering on pedigree to account for family relationships) and for whether the DNA had been whole genome amplified. In a second step we additionally adjusted our most significant associations both for systolic blood pressure and for the presence or absence of hypertension (defined using criteria from the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg or use of antihypertensive medications).[21] All four studies screened for latent population substructure, which was negligible.

Meta-analysis

We conducted a meta-analysis of results from the four cohorts using inverse-variance weighting, also known as fixed-effects meta-analysis. Prior to the meta-analysis, strand alignment was verified across all studies. After QC, filtering, and imputation within each study, we restricted our meta-analysis to the 2,194,468 autosomal SNPs that were common to all studies. We decided a priori on a genome-wide significance threshold of $5x10^{-8}$ which corresponds to a target α(p-value) of 0.05 with a Bonferroni correction for 1 million independent tests. For 2.2 million tests, it corresponds to an expectation of only 0.11 false positives, regardless of test-dependence. The linkage disequilibrium pattern seen in ongoing deep sequencing efforts within European populations also supports the use of this threshold.[22] SNPs with $5x10^{-8}<p<1x10^{-5}$ were considered highly suggestive associations, but not genome-wide significant; SNPs with $1x10^{-5}<p<1x10^{-4}$ were considered only moderately suggestive associations.

Replication

We undertook in-silico replication of the findings for which we obtained genome-wide significance in the CHARGE cohort in an independent sample, the African-American participants in the ARIC study.[10] We studied 2430 persons (889 men; mean age: 53±6 years)

who were initially free of stroke and consented for genotyping. Stroke definition and surveillance methods in this replication sample were identical to that used in white ARIC participants.[20] Genotyping was undertaken using the Affymetrix GeneChip® SNP Array 6.0. Imputation was not attempted as current HapMap release versions do not have linkage disequilibrium data in African-Americans, but only in Africans. Only one of the two top snps was present on the array used and was investigated using additive genetic models. The CHS had genotyping on 574 African Americans, but the platform used did not genotype the high signal SNPs.

RESULTS

We observed 1,544 incident stroke events (1,164 ischemic strokes) among our study sample of 19,602 persons followed for an average of 11 years (Table 1). Figure 1A shows the genome-wide plot of p-values for the individual SNPs against their genomic position for total stroke, and Figure 1B for ischemic stroke. For highly suggestive loci with $p<1x10^{-5}$, the hazard ratios and population attributable risks associated with the minor allele are presented in Table 2. At a threshold of $p<1x10^{-4}$ (i.e. moderately suggestive), a total of 347 SNPs were associated with total stroke and 256 SNPs with ischemic stroke.

Two SNPs at one locus surpassed our preset threshold ($p=5x10^{-8}$) for genome-wide significance both for total stroke and ischemic stroke (Table 2). Despite the smaller number of events in the analyses of ischemic stroke the hazard ratios were slightly larger and p-values slightly smaller for the association of these two SNPs with ischemic stroke than in the analysis of total stroke. Each copy of a minor allele at these loci increased the hazard ratio for total stroke by 31 to 32% (95% CI: 19-44) and for ischemic stroke by 39 to 41% (95% CI: 27-56%). The corresponding population attributable risks are 11-13% for total stroke and 14-17% for ischemic stroke. For strokes other than confirmed ischemic strokes (i.e. hemorrhagic and unknown types) we found no effect (hazard ratio=1.13; 95%CI: 0.94-1.36; p=0.20); thus the findings for total stroke reflect the strong association with ischemic stroke. The risk estimates for both SNPs were similar across the four studies as shown in the Forest plots (Figure 2A to D). Both SNPs were in significant linkage disequilibrium with each other ($r^2 = 0.73$ based on HapMap CEU data, NCBI build #36). Figure 3 shows all SNPs within a 200kbp region on either side of these two SNPs, together with the recombination rates and the known genes in that region. Adjustments for systolic blood pressure or hypertension had negligible effects on the associations.

We were able to replicate the association of the first SNP in the African-American sample. Over a follow-up period of 15 years, 215 persons suffered an incident stroke and 191 of these were ischemic. The first SNP, with a minor allele frequency of 10%, was associated with incident total stroke (HR=1.39;95% CI:1.05-1.84;p=0.02) and incident ischemic stroke

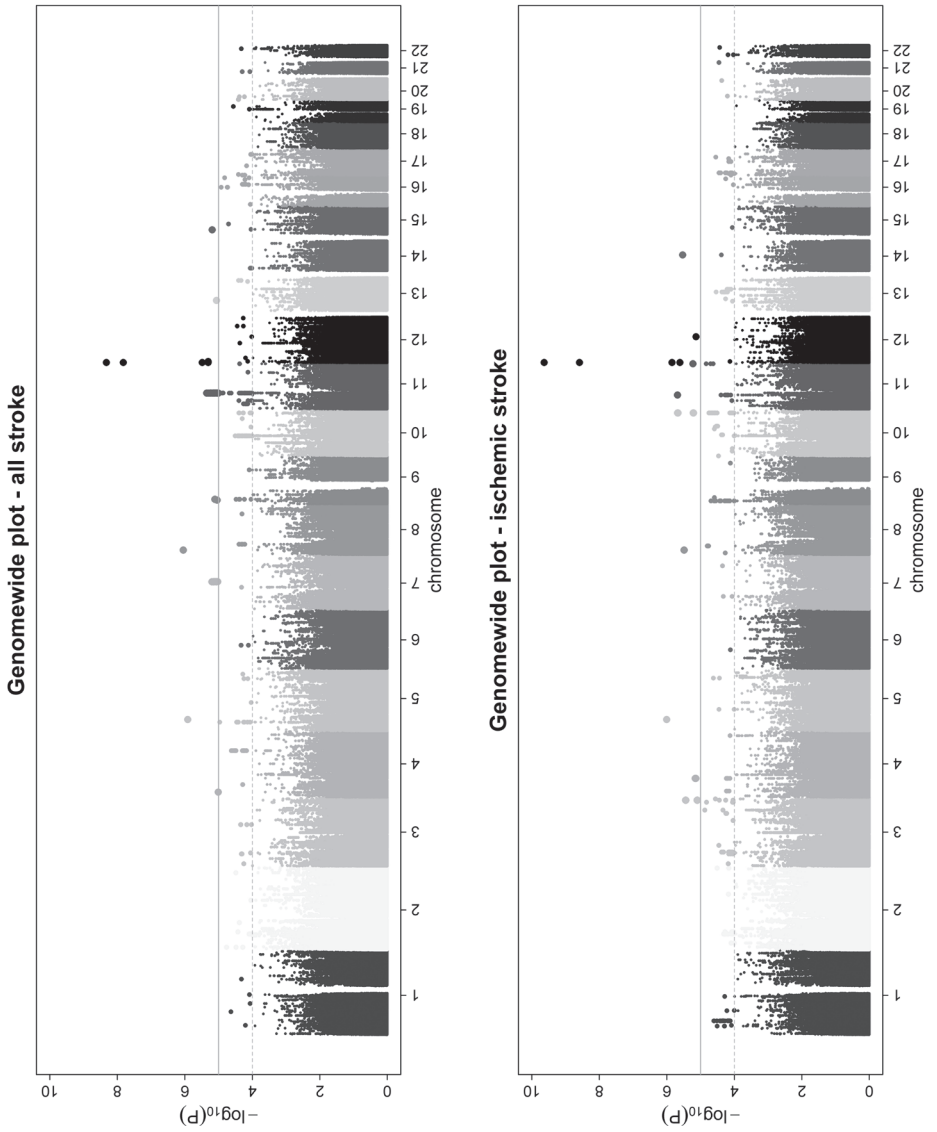

Figure 1A and B. Genome-wide signal intensity (Manhattan) plots showing the individual p-values (based on the fixed-effects meta-analysis) against their genomic position for total stroke (A - top) and ischemic stroke (B - bottom). Within each chromosome, shown on the x-axis, the results are plotted left to right from the p-terminal end. The solid line indicates the threshold for $p=10^{-5}$. Hits above this line are shown in Table 2. The dashed line indicates the threshold for $p=10^{-4}$.

(HR=1.43;95% CI:1.06-1.92;p=0.02).with population attributable risks among African-Americans of 7% and 8%, respectively, per copy of the minor allele.

Table 2. Strongest single nucleotide polymorphism (SNP)-phenotype associations at meta-analysis for total stroke and ischemic stroke

SNP nr	Minor allele	MAF	Hazard ratio	p-value	PAR	Additional SNPs at locus with $p<10^{-5}$
			Total stroke			
1	A	0.23	1.32 (1.20; 1.44)	$4.8x10^{-9}$	0.13	3
2	A	0.19	1.31 (1.19; 1.44)	$1.5x10^{-8}$	0.11	3
3	T	0.47	1.23 (1.13; 1.33)	$9.1x10^{-7}$	0.18	
4	A	0.11	1.41 (1.23; 1.62)	$1.2x10^{-6}$	0.09	
5	T	0.22	1.23 (1.13; 1.35)	$4.4x10^{-6}$	0.10	49
6	G	0.20	1.31 (1.17; 1.48)	$4.9x10^{-6}$	0.12	
7	G	0.45	0.83 (0.77; 0.90)	$6.4x10^{-6}$	§	14
8	T	0.45	1.22 (1.12; 1.33)	$6.5x10^{-6}$	0.17	
9	G	0.39	1.20 (1.11; 1.29)	$7.8x10^{-6}$	0.14	1
10	C	0.06	1.44 (1.23; 1.69)	$8.7x10^{-6}$	0.05	
11	G	0.09	1.32 (1.27; 1.49)	$9.8x10^{-6}$	0.06	
			Ischemic stroke			
1	A	0.23	1.41 (1.27; 1.56)	$2.3x10^{-10}$	0.17	3
2	A	0.19	1.39 (1.25; 1.54)	$2.6x10^{-9}$	0.14	3
3	A	0.11	1.49 (1.27; 1.75)	$9.9x10^{-7}$	0.10	
4	A	0.29	0.78 (0.70; 0.85)	$2.1x10^{-6}$	§	
5	T	0.43	1.24 (1.13; 1.35)	$2.1x10^{-6}$	0.18	3
6	A	0.27	0.75 (0.66; 0.85)	$3.0x10^{-6}$	§	
7	T	0.47	1.26 (1.14; 1.38)	$3.3x10^{-6}$	0.20	
8	G	0.06	1.49 (1.26; 1.76)	$3.6x10^{-6}$	0.05	1
9	G	0.16	1.36 (1.19; 1.55)	$6.0x10^{-6}$	0.10	
10	A	0.28	1.24 (1.13; 1.36)	$6.9x10^{-6}$	0.12	2
11	C	0.09	1.53 (1.27; 1.84)	$7.4x10^{-6}$	0.09	

P-values, hazard ratios and 95% confidence intervals (CI) are based on fixed-effects (inverse variance-weighted) meta-analysis. Each row specifically identifies only the SNP-phenotype association with the lowest p-value for that locus, except for the two associations reaching genome-wide significance that are both shown here. SNPs highlighted in bold were associated with both total stroke and ischemic stroke. The last column shows the number of additional SNPs at the same locus, within 250kb of the specified SNP, that were also associated with the phenotype with a p-value $<10^{-5}$. Complete details for these additional SNPs are provided in the Supplementary Tables A and B available online.

*Alleles were identified based on the plus strand of the NCBI build #36. The minor allele was also the coded allele; MAF: Minor allele frequency is based on allele frequency in meta-analysis sample.

PAR: Population attributable risk

§ Population attributable risks are not reported for these minor alleles since they were protective.

We also examined associations with SNPs reported to be significantly associated with either total stroke or ischemic stroke in published meta-analysis; thus we examined SNPs on the following genes: *MTHFR, F5, PDE4D, SERPINE1, F2, GP1BA, ALOX5AP, APOE and ACE*. Rs16954257 in *GPIBA*, the glycoprotein 1 b alpha gene, a proxy SNP for rs2243093 previously associated with stroke,[23] was significantly associated with all stroke (p=0.02) and ischemic stroke (p=0.02) in our meta-analysis. For prothrombin (*F2*) the previously reported SNP was not present in our imputed dataset.[24] However, six other SNPs at that locus reached $p<10^{-4}$ for total stroke.

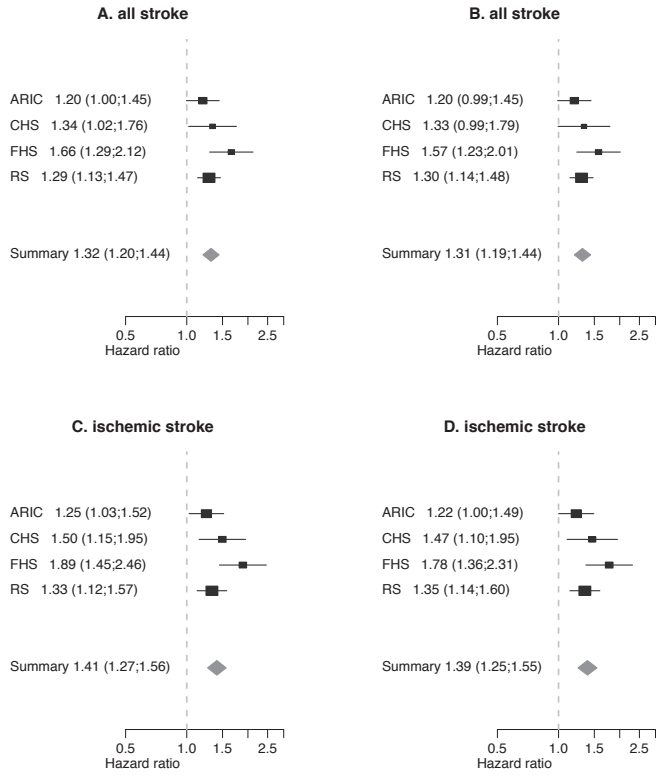

Figure 2: Forest plots for the first (A and C) and second (B and D) top SNP. Individual studies (boxes) are plotted against the individual effect sizes (hazard ratios). The size of the box is inversely proportional to the variance. Horizontal lines are the 95% confidence interval. The y-axis shows the value for no effect (hazard ratio=1). Note that the x-axis is on a logarithmic scale.

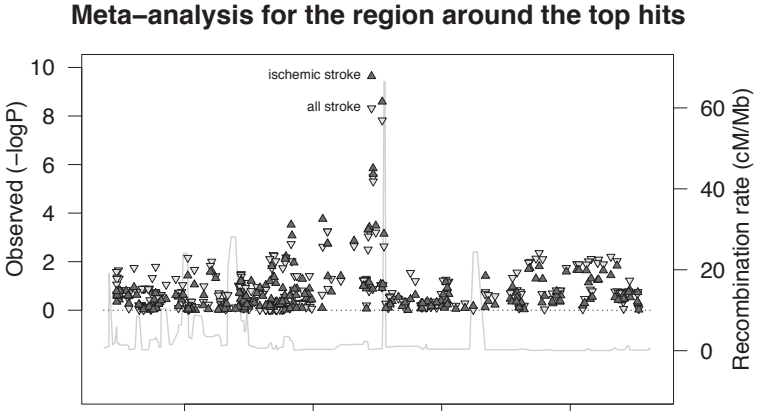

Figure 3: Regional plot for associations in the region of 200 kbp on either side of the top SNPs. All SNPs are plotted with their meta-analysis p-values against their genomic position. The triangles pointing down show meta-analysis p-values for total stroke, and the triangles pointing up for ischemic stroke. The light line represents the estimated recombination rates.

DISCUSSION

In this meta-analysis of GWAS data from four large cohort studies of incident stroke events, two previously unsuspected common SNPs were consistently associated with total stroke and ischemic stroke in persons of European descent. We were able to replicate this finding in an independent sample of persons of African-American ethnicity.

We had a priori considered total stroke, a heterogenous phenotype, as our primary analysis, but the finding that the association of our top SNPs was stronger for the phenotype of ischemic stroke, despite the smaller sample size than total stroke, and that the association was absent in the non-ischemic subgroup, indicates that these SNPs were primarily associated with ischemic stroke. The effect sizes were similar in all four discovery cohorts and in the replication cohort, and the minor alleles were associated with an increased ischemic stroke risk of 31-43% per copy of the allele.

Several of the highly suggestive SNP-phenotype associations were in or adjacent to genes that may be biologically plausible candidates to affect stroke risk since they modulate inflammation and coagulation, synaptic function, neuronal survival or cell adhesion). These highly suggestive findings for these genes need further examination in other studies.

The strengths of this study include the community-based prospective design, the large sample of incident cases of both fatal and non-fatal stroke, the high-quality of methods used to identify and classify stroke, and the ongoing surveillance that verifies cohort members serving as controls have remained free of stroke; these strengths extend to both the discovery and replication cohorts. These associations are unlikely to be due to population stratification since the discovery sample was restricted to whites of European origin and was also investigated for latent population substructure. Moreover, we were able to replicate our significant locus in an independent sample of different ethnicity.

The study also has limitations. The identified intergenic SNPs may not represent the causal variants but are expected to be in linkage disequilibrium with the causal variants, which remain to be uncovered. Further exploration of this genomic region with dense genotyping, expression and translational studies will be required. There were too few events to study genotype-phenotype associations underlying individual subtypes of ischemic stroke, such as cortical or lacunar ischemic strokes. Finally, we had limited power to detect associations with small effect sizes and associations with rare variants.

In conclusion, this meta-analysis of GWAS data from four large community-based studies has identified previously unsuspected associations with incident stroke. Exploring the epidemiological correlates and the molecular, cellular and clinical consequences of genetic variation at this locus may yield novel insights into the pathophysiology of stroke.

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Chapter 4.2

The GAB2 Gene and the Risk of Alzheimer's Disease: Replication and Meta-analysis

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ABSTRACT

Background: In a recent genome-wide association study, the GAB2-gene has been suggested to modify the risk of late-onset Alzheimer's disease (AD) among APOEε4-carriers. However, replication data are scarce and inconsistent.

Methods: In a population-based cohort study (n=5,507; age>55) with 443 incident AD cases we sought to replicate the association between rs4945261 and AD. Because we used high-density genotyping of GAB2, we also investigated several other polymorphisms within and around this gene. Furthermore, we performed a meta-analysis with all previously published studies.

Results: We found that rs4945261 was associated with AD among APOEε4-carriers (p=0.02), but not among non-carriers (p=0.26). Fifteen of the 20 remaining polymorphisms within GAB2 and several polymorphisms in the 250kbp-region surrounding GAB2 were also associated with AD among APOEε4-carriers and only one among non-carriers. For rs2373115 meta-analysis with published studies yielded an odds-ratio of 1.58 (1.17-2.14) with $p=3.0*10^{-3}$ among APOEε4-carriers and 1.09 (0.97-1.23) with p=0.16 among non-carriers. For rs4945261 the pooled odds-ratio was 1.75 (1.21-2.55) with $p=3.0*10^{-3}$ among APOEε4-carriers and 1.20 (1.01-1.41) with p=0.03 among non-carriers.

Conclusions: We found the GAB2 gene to be associated with AD. When taken together with published data, our data suggest GAB2 to modify the risk of AD in APOEε4-carriers.

INTRODUCTION

The quest for finding genes that are related to Alzheimer's disease (AD) has turned towards using high throughput genotyping analysis, in which thousands of polymorphisms can be studied concomitantly. Several genome-wide association studies have been conducted for AD so far and most found a consistent and strong hit in or around the Apolipoprotein E (*APOE*) gene, confirming earlier linkage and candidate gene studies.[1-3] Reiman et al. carried out a genome-wide association analysis after stratification by the *APOE*ε4-allele.[4] They showed that the gene encoding GRB-associated binding protein 2 (*GAB2*) was associated with AD in *APOE*ε4-carriers, but not in non-carriers. Replication data are scarce and inconsistent: Chapuis et al.[5] failed to find an association between *GAB2* with AD in three independent study samples, either in *APOE*ε4-carriers or non-carriers. Li et al.[3] also did not confirm this finding in their genome-wide association study, but did not stratify by *APOE*ε4-status. However, in a Belgian sample Sleegers et al.[6] did find an association, and finally Miyashita et al.[7] could not replicate this association in a Japanese population.

In the population-based Rotterdam Study, we sought to replicate the association between *GAB2* and AD. We investigated rs4945261, which was one of the SNPs in the original report. Moreover, because we used high-density genotyping, we also investigated other SNPs within *GAB2* and within a 250kbp-region surrounding the gene. Finally, we meta-analyzed our results with those from previously published studies.

METHODS AND MATERIALS

Study population

The Rotterdam Study is a prospective population-based cohort study of 7,983 Caucasian participants (aged 55 years and over) living in Ommoord, a district of Rotterdam, The Netherlands.[8] The study investigates determinants of chronic diseases in the elderly, including AD. Persons gave written informed consent to participate and the study was approved by the institutional medical-ethics committee. At baseline (1990-1993) participants were interviewed and underwent physical examination and blood sampling. For the present study, only persons who were non-demented at baseline were eligible (n=7,046). No overlap exists between our study population and the Dutch sample reported on in the original report.[4]

Genotyping

Only participants with proper quality DNA-samples (n=6,449) were considered for genotyping using the version 3 Illumina-Infinium-II HumanHap550SNP chip-array as part of a large project on genetics of complex diseases. Genotyping procedures were followed according to manufacturer's protocol.[9] After quality control 5,974 persons remained with proper genotyped data. No population stratification was present in this sample.[9] For the current report we extracted data on rs4945261. We also extracted other SNPs that were located in *GAB2* (total of 20 SNPs) or within a 250-kbp region surrounding the gene (94 SNPs), and were in Hardy-Weinberg equilibrium ($p>0.001$). In order to pool our data with all previous studies we imputed allelic data for rs2373115 based on the local linkage disequilibrium structure using the MACH-imputation software (http://www.sph.umich.edu/csg/abecasis/MACH). The quality of imputation was 99.8%.

APOE genotyping was performed on coded samples without knowledge of the other measurements as described elsewhere,[10] and was unavailable in 467 persons mostly due to technical reasons leaving a total of 5,507 persons available in the current analysis.

Ascertainment of incident AD

The diagnosis of incident AD was made following a three-step protocol.[11] At baseline (1990-1993) and during three follow-up visits (1993-1994, 1997-1999, 2002-2004) two brief tests of cognition (MMSE and Geriatric Mental State schedule (GMS)) were used to screen all subjects. Screen-positives (MMSE score<26 or GMS>0) underwent the Cambridge examination for mental disorders of the elderly (Camdex). Persons suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for diagnosis. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident AD through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional

Institute for Outpatient Mental Health Care. The diagnosis of AD was made in accordance with internationally accepted criteria by a panel of a neurologist, neuropsychologist and research physician. Follow-up was complete until January 1st, 2005.

Statistical analysis

We used the allelic χ^2-test with one degree-of-freedom to investigate the association between rs4945261 and AD, before and after stratification by *APOE*ε4-status. We used a threshold of p=0.05 for statistical significance, because our aim was to replicate previous genome-wide findings.

A similar approach was used when investigating the remaining 20 SNPs within *GAB2*. However, in this instance we also assessed multiple testing by calculating false-discovery rates[12] and by permutation testing. Subsequently, we analyzed the 94 SNPs in the region surrounding *GAB2*.

We further explored these associations by using Cox'-proportional hazards models and adjusting for age, sex, and time-to-event. Finally, we tested for interaction by adding an interaction term SNP**APOE*ε4-status to the models.

For our meta-analysis strategy, see the Appendix.

RESULTS

Table 1 shows the characteristics of the study population. *APOE*ε4-carriers were younger than non-carriers and as expected had a shorter follow-up time with a larger percentage of incident AD cases.

Table 1. Characteristics of the study population.

	Total	*APOE*ε4 non-carriers	*APOE*ε4 carriers	p-value
N	5,507	3,958	1,549	
Age	68.9 (8.7)	69.1 (8.8)	68.4 (8.4)	0.01*
Women	3,215 (58%)	2,322 (59%)	893 (58%)	0.67**
Mean follow-up	9.24 (3.21)	9.31 (3.17)	9.07 (3.32)	<0.01†
Incident AD cases	443 (8%)	249 (6%)	194 (13%)	<0.01†

Values are numbers (percentages) or means (standard deviation). The p-values are for the difference between *APOE*ε4 carriers and non-carriers. AD: Alzheimer's disease

* sex-adjusted

** age-adjusted

† age and sex-adjusted

Table 2 shows the association of SNPs in *GAB2* with AD. Rs4945261, the only SNP similar to the discovery report, showed a significant association with AD (p=0.02). Stratification by the *APOE*ε4-allele showed that the association was particularly marked in carriers of the *APOE*ε4-allele. In non-carriers no significant association with AD was seen.

Table 2. Association between polymorphisms in *GAB2* and Alzheimer's disease.

SNP	Position	MA	MAF	RA	Overall		*APOE*ε4 non-carriers		*APOE*ε4 carriers	
					p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)
rs2450135	77605643	A	0.03	G	0.06	0.73 (0.52-1.02)	0.16	0.73 (0.47-1.13)	0.17	0.69 (0.41-1.17)
rs1318241	77608440	A	0.14	G	0.02	1.28 (1.04-1.59)	0.27	1.16 (0.89-1.53)	0.02	1.51 (1.08-2.11)
rs2450129	77618033	G	0.15	A	0.02	1.29 (1.04-1.59)	0.27	1.17 (0.89-1.53)	0.01	1.52 (1.09-2.13)
rs731600	77640781	G	0.15	A	0.02	1.28 (1.04-1.58)	0.27	1.17 (0.89-1.53)	0.02	1.50 (1.07-2.08)
rs1893447	77650830	G	0.15	A	0.02	1.29 (1.05-1.59)	0.24	1.18 (0.90-1.54)	0.01	1.52 (1.09-2.12)
rs2511175	77652729	G	0.15	A	0.02	1.28 (1.04-1.58)	0.26	1.17 (0.89-1.54)	0.02	1.49 (1.07-2.07)
rs1981405	77653856	A	0.11	G	0.04	1.29 (1.01-1.64)	0.22	1.22 (0.89-1.67)	0.06	1.44 (0.99-2.09)
rs7927923	77657062	G	0.19	A	0.41	1.08 (0.90-1.29)	0.97	1.00 (0.80-1.26)	0.18	1.21 (0.92-1.61)
rs4945261	**77667908**	**A**	**0.15**	**G**	**0.02**	**1.28 (1.04-1.58)**	**0.26**	**1.17 (0.89-1.54)**	**0.02**	**1.49 (1.07-2.07)**
rs7107174	77675584	A	0.14	G	0.02	1.28 (1.03-1.58)	0.26	1.17 (0.89-1.54)	0.02	1.48 (1.06-2.07)
rs4944196	77686379	A	0.15	G	0.02	1.29 (1.04-1.59)	0.19	1.20 (0.91-1.58)	0.03	1.45 (1.05-2.02)
rs6592772	77693211	C	0.15	A	0.02	1.29 (1.05-1.60)	0.20	1.19 (0.91-1.57)	0.02	1.49 (1.07-2.08)
rs10899469	77695961	G	0.15	A	0.02	1.29 (1.04-1.59)	0.23	1.18 (0.90-1.55)	0.02	1.50 (1.08-2.09)
rs11237451	77703107	G	0.19	A	0.29	1.10 (0.92-1.32)	0.65	1.06 (0.84-1.34)	0.22	1.19 (0.90-1.57)
rs2292572	77730512	A	0.15	C	0.01	1.32 (1.07-1.63)	0.13	1.23 (0.94-1.62)	0.01	1.51 (1.09-2.09)
rs10501426	77734770	A	0.15	G	0.01	1.31 (1.07-1.62)	0.13	1.23 (0.94-1.62)	0.02	1.48 (1.07-2.05)
rs11601726	77745687	G	0.12	A	0.95	1.01 (0.82-1.24)	0.80	0.96 (0.73-1.27)	0.72	1.06 (0.76-1.48)
rs11603112	77751139	A	0.15	G	0.01	1.34 (1.08-1.65)	0.14	1.23 (0.94-1.62)	0.01	1.55 (1.11-2.16)
rs2373115	***77768798***	***A***	***0.15***	***C***	***0.01***	***1.32 (1.07-1.63)***	***0.10***	***1.26 (0.96-1.65)***	***0.02***	***1.46 (1.06-2.01)***
rs7112234	77780118	A	0.15	G	0.01	1.33 (1.08-1.64)	0.10	1.26 (0.96-1.66)	0.02	1.47 (1.07-2.04)
rs7941639	77794607	A	0.14	G	0.03	1.26 (1.02-1.57)	0.42	1.12 (0.85-1.48)	0.02	1.53 (1.08-2.17)
rs10899496	77801479	G	0.15	A	0.01	1.34 (1.09-1.65)	0.08	1.28 (0.97-1.68)	0.02	1.48 (1.07-2.05)

Odds ratios are unadjusted and calculated using the allelic χ^2 test.

MA Minor allele, MAF Minor allele frequency, RA Risk allele, OR odds ratio, CI Confidence interval.

SNPs in bold were also genotyped by Reiman et al[4].The SNP in italic was imputed.

Of the 20 remaining SNPs in *GAB2* 15 also showed a significant (p<0.05) association as well as the imputed SNP rs2373115 (Table2). Table 3 shows that although the p-values would not survive multiple-testing correction for 21 SNPs, the probability that these findings are false-discoveries is very small. In the Figure p-values for all SNPs within 250-kbp of the *GAB2* gene are plotted against their respective genomic position and stratified by *APOE*ε4-allele. Among *APOE*ε4-carriers various SNPs that were in high linkage disequilibrium (LD) with SNPs within *GAB2* had a p-value<0.05. Non-significant SNPs were located further away from *GAB2* across recombination sites and in other LD-blocks (see Figure). In contrast, in non-carriers only one SNP in the whole region located outside *GAB2* had a p-value<0.05.

Table 4 shows hazard-ratios, adjusted for age, sex and time-to-event. The associations among non-carriers hardly changed, but among *APOE*ε4-carriers the associations attenuated slightly. Nevertheless, eleven of the 22 SNPs were still significant among *APOE*ε4-carriers, whereas several others SNPs were borderline significant. Finally, the interaction term for *APOE*ε4*SNP was not significant for any SNP (data not shown).

Table 3. Statistics for the association between polymorphisms in *GAB2* and incident Alzheimer's disease, stratified by *APOE* ε4 status.

A. *APOE* ε4 carriers				
SNP	Unadjusted p-value	False discovery rate*	Adjusted False discovery rate**	Corrected empirical p-value***
rs11603112	0.0109	0.0290	0.0050	0.0672
rs1893447	0.0134	0.0290	0.0050	0.0787
rs2450129	0.0141	0.0290	0.0050	0.0821
rs2292572	0.0147	0.0290	0.0050	0.0866
rs1318241	0.0162	0.0290	0.0050	0.0918
rs10899469	0.0164	0.0290	0.0050	0.0922
rs7941639	0.0176	0.0290	0.0050	0.0941
rs731600	0.0177	0.0290	0.0050	0.0963
rs10501426	0.0184	0.0290	0.0050	0.0978
rs10899496	0.0184	0.0290	0.0050	0.0978
rs6592772	0.0185	0.0290	0.0050	0.0986
rs2511175	0.0194	0.0290	0.0050	0.1036
rs4945261	0.0194	0.0290	0.0050	0.1036
rs7112234	0.0199	0.0290	0.0050	0.1042
rs7107174	0.0207	0.0290	0.0050	0.1160
rs4944196	0.0257	0.0337	0.0058	0.1374
rs1981405	0.0574	0.0709	0.0121	0.2652
rs2450135	0.1710	0.1976	0.0341	0.6026
rs7927923	0.1788	0.1976	0.0356	0.6276
rs11237451	0.2231	0.2343	0.0440	0.6833
rs11601726	0.7215	0.7215	0.1295	0.9992

B. *APOE* ε4 non-carriers				
SNP	Unadjusted p-value	False discovery rate*	Adjusted False discovery rate**	Corrected empirical p-value***
rs10899496	0.0824	0.3365	0.0995	0.3366
rs7112234	0.0954	0.3365	0.0995	0.3750
rs10501426	0.1303	0.3365	0.0995	0.4728
rs2292572	0.1337	0.3365	0.0995	0.4817
rs11603112	0.1382	0.3365	0.0995	0.4883
rs2450135	0.1568	0.3365	0.0995	0.5417
rs4944196	0.1916	0.3365	0.0995	0.6258
rs6592772	0.2060	0.3365	0.0995	0.6435
rs1981405	0.2151	0.3365	0.0995	0.6605
rs10899469	0.2370	0.3365	0.0995	0.6921
rs1893447	0.2426	0.3365	0.0995	0.6965
rs7107174	0.2569	0.3365	0.0995	0.7329
rs4945261	0.2574	0.3365	0.0995	0.7330
rs2511175	0.2597	0.3365	0.0995	0.7337
rs731600	0.2664	0.3365	0.0995	0.7416
rs2450129	0.2665	0.3365	0.0995	0.7423
rs1318241	0.2724	0.3365	0.0995	0.7642
rs7941639	0.4175	0.4870	0.1448	0.9150
rs11237451	0.6476	0.7157	0.2080	0.9937
rs11601726	0.8011	0.8412	0.2452	0.9999
rs7927923	0.9743	0.9743	0.2832	1.0000

Statistics are based on the 21 *genotyped* SNPs in *GAB2*. SNPs are ordered by the unadjusted p-value

* Using the method described by Benjamini and Hochberg.[12]

** calculated using the R-package 'fdrtool',[17] which additionally adjusts for the estimated proportion of true null associations

*** obtained after 10,000 permutations

Meta-analysis with published studies showed a pooled random-effects odds ratio for rs2373115 among *APOE*ε4-carriers of 1.58 (95%CI 1.17-2.14) with $p=3.0*10^{-3}$. For rs4945261 the meta-analysis showed among *APOE*ε4-carriers a random-effects odds ratio of 1.75 (1.21-2.55) with $p=3.0*10^{-3}$. Among non-carriers the odds ratios were 1.09 (0.97-1.23) with p=0.16 for rs2373115 and 1.20 (1.01-1.41) with p=0.03 for rs4945261 (see Appendix).

DISCUSSION

In this population-based cohort study we found that rs4945261 was associated with AD in persons carrying the *APOE*ε4-allele. In non-carriers no significant association was found. Furthermore, we also found that several other SNPs within and around *GAB2* were associated with AD in *APOE*ε4-allele carriers but not in non-carriers, though these would not survive multiple-testing correction.

Table 4. Association between polymorphisms in *GAB2* and Alzheimer's disease using Cox'-proportional hazards models.

SNP	Position	Hazard ratios (95% CI)	
		*APOE*ε4 non-carriers	*APOE*ε4 carriers
rs2450135	77605643	0.74 (0.50-1.11)	0.76 (0.48-1.20)
rs1318241	77608440	1.16 (0.90-1.51)	1.38 (1.00-1.90)
rs2450129	77618033	1.17 (0.90-1.52)	1.38 (1.00-1.90)
rs731600	77640781	1.17 (0.90-1.51)	1.36 (0.99-1.87)
rs1893447	77650830	1.17 (0.90-1.51)	1.37 (1.00-1.88)
rs2511175	77652729	1.17 (0.90-1.52)	1.36 (0.99-1.87)
rs1981405	77653856	1.22 (0.90-1.64)	1.32 (0.92-1.88)
rs7927923	77657062	0.99 (0.79-1.23)	1.11 (0.86-1.44)
rs4945261	**77667908**	**1.17 (0.90-1.52)**	**1.36 (0.99-1.87)**
rs7107174	77675584	1.17 (0.90-1.53)	1.35 (0.99-1.85)
rs4944196	77686379	1.20 (0.92-1.55)	1.32 (0.97-1.80)
rs6592772	77693211	1.20 (0.92-1.55)	1.37 (0.99-1.88)
rs10899469	77695961	1.18 (0.91-1.54)	1.38 (1.00-1.89)
rs11237451	77703107	1.03 (0.82-1.29)	1.10 (0.85-1.42)
rs2292572	77730512	1.21 (0.93-1.57)	1.38 (1.02-1.88)
rs10501426	77734770	1.21 (0.93-1.57)	1.36 (1.01-1.85)
rs11601726	77745687	0.97 (0.74-1.26)	1.14 (0.84-1.55)
rs11603112	77751139	1.19 (0.92-1.55)	1.41 (1.03-1.92)
rs2373115	***77768798***	***1.23 (0.95-1.61)***	***1.36 (1.00-1.85)***
rs7112234	77780118	1.23 (0.95-1.61)	1.36 (1.01-1.85)
rs7941639	77794607	1.12 (0.86-1.45)	1.44 (1.04-2.00)
rs10899496	77801479	1.24 (0.95-1.61)	1.37 (1.01-1.86)

Hazard ratios are adjusted for age, sex, and time to event.

CI Confidence interval

SNPs in bold were also genotyped by Reiman et al.[4] The SNP in italic was imputed.

To our knowledge this is the first study to longitudinally investigate the association between *GAB2* and AD. The population-based design limits the possibility of selection biases often seen in case-control studies. A possible limitation is that in some cases the diagnosis of AD might have been misclassified. However, such misclassification is likely to be random and would therefore lead to an underestimation of the true effect. Another consideration is that apart from rs4945261 the other SNPs were different from previous reports. However, additional genotyping is unlikely to change our results given the density of SNPs we studied and the low recombination rate in *GAB2* (see Figure). Moreover, genotyping different SNPs can be regarded as contributing to fine-mapping the *GAB2*-gene and its association with AD. Our associations would not have survived stringent multiple testing correction for 21 SNPs. However, given the strong LD between SNPs and the low prior probability of these findings being false-positive, standard multiple testing could be considered overly conservative. More importantly, the meta-analysis also points towards a positive association.

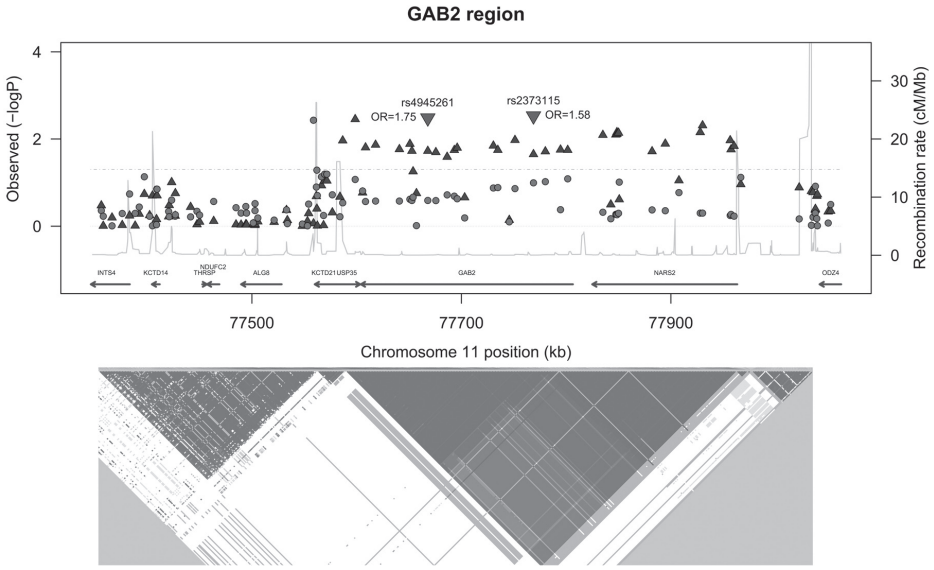

Figure. The association between polymorphisms surrounding the *GAB2* gene and Alzheimer's disease.
P-values are for the allelic χ^2-test. Triangles pointing up indicate p-values for *APOE*ε4 carriers. Circles indicate p-values for *APOE*ε4 non-carriers. Large triangles pointing down indicate p-values for the meta-analysis in *APOE*ε4 carriers. The dot-dashed line indicates the threshold for p-value=0.05. Light line indicates the estimated recombination rates (as obtained from HapMap) reflecting the local linkage disequilibirum (LD) structure. Known genes (arrows) are aligned along their genomic position.
At the bottom the LD structure is shown as obtained from HapMap (for the CEU-population). Light colors indicate low LD, darker colors indicate high LD.

Note that the p-values at the lower and upper end of the figure are similar between *APOE*ε4 carriers and non-carriers, but that they diverge in the LD-block encompassing *GAB2*.

Thus far, three studies have failed to replicate the initial findings[3,5,7] and only one confirmed the association.[6] In line with the initial study, we found that *GAB2* alleles were associated with AD only among *APOE*ε4-carriers, and not in non-carriers. Pooling our data with previously published data showed highly significant associations with odds ratios of 1.58 and 1.75.

GAB2 is a protein involved in various pathways, some of which involve AD-related tau processing.[13-15] Indeed, Reiman et al. also found that *GAB2* expression was associated with protection from neurofibrillary tangle formation.[13-15] Moreover, *GAB2* is expressed together with other potential AD-related genes.[16] However, the exact mechanism of interaction with the *APOE*-gene is still unknown. Future research should focus on disentangling the exact interactive mechanism as well as high-density sequencing of *GAB2* to find the possible causative variant.

In conclusion, we found *GAB2* to be associated with AD. Together with previous data, this suggests *GAB2* as a novel gene modifying the risk of AD in *APOE*ε4-carriers.

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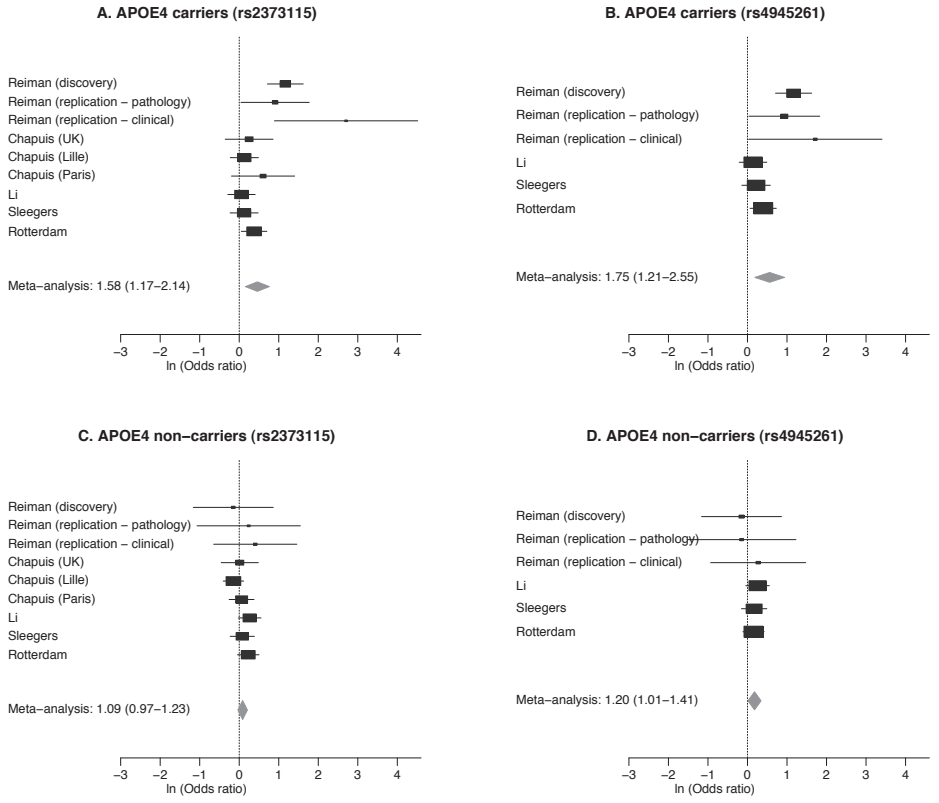
Appendix to Chapter 4.2

METHODS FOR META-ANALYSIS

We conducted a meta-analysis using random-effects pooling for rs4945261 and rs2373115 based on data from previously published studies.[1-4] To ensure we did not miss any other study we searched PubMed using the key-words GAB2, Alzheimer's disease, and dementia. We also sought through reference lists of previous papers and queried the AlzGene database (www.alzgene.org). We did not find any other studies and restricted our current meta-analysis to Caucasian populations.



Supplementary figure. Meta-analysis of published studies on the association between polymorphisms in *GAB2* and Alzheimer's disease, stratified by the *APOE* ε4 allele.

From all studies, genotype frequencies were obtained for cases and controls and the odds ratios calculated using the allelic χ^2-test with one degree-of-freedom. No additional adjustments were made.The effect sizes (boxes) with 95% confidence intervals are plotted.

The size of the box is proportional to the weight of the study. The diamond is for the random-effects meta-analysis. Note that the x-axis is depicted on a logarithmic scale.

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Part 5

Chapter 5.1

General Discussion

In this thesis I investigated genetic and non-genetic determinants and clinical outcomes of structural brain changes on MRI. I did not investigate specific focal markers, but instead I adopted a general approach in which the focus was on markers across the whole brain and the whole genome. In this section I will review the main findings, discuss general methodological considerations, and consider implications of the findings with respect to clinical practice and future research.

MAIN FINDINGS

Descriptives and risk factors of structural brain changes

Several neuro-imaging studies have investigated brain tissue volume changes across various broad age-ranges.[1-5] However, population data on brain volume changes in the elderly were scarce. In chapter 2.1.1 we quantified brain tissue volumes in persons aged 60 to 90 and found that white matter atrophy rather than grey matter atrophy seemed to drive loss of brain volume with increasing age. We also found that classic cardiovascular risk factors (blood pressure, hypertension, and smoking), white matter lesions and lacunar infarcts were more strongly associated with white matter atrophy than grey matter atrophy. These results fit well with pathologic studies showing that grey matter decline in healthy elderly brains is minimal.[6, 7] Moreover, in recent years a substantial body of literature is emerging indicating that white matter atrophy may be more important in aging and neuro-degeneration than thus far recognized.[8-13] Various neuro-imaging as well as pathologic studies suggest that white matter atrophy is reflective of the same pathologic process as white matter lesions and lacunar infarcts, namely cerebral small vessel disease.[8, 9, 14-21] In turn, cardiovascular risk factors are thought to be etiologically linked with cerebral small vessel disease.[12, 22-25] Therefore, our results that cardiovascular risk factors, white matter lesions, and lacunar infarcts are strongly associated with white matter atrophy provide further evidence for a vascular basis of white matter atrophy. These findings prompted us to further investigate brain volume changes, especially white matter atrophy, with regard to novel putative risk factors and clinical correlates.

We investigated kidney disease and unrecognized myocardial infarction as putative risk factors of vascular brain disease. Both are highly prevalent in the general population, and have been shown to indicate a poor prognosis for various clinical outcomes.[26-33] Another feature of these risk factors is that both reflect subclinical vascular disease, in the kidney and heart respectively. In line with previous findings,[34-36] we found that persons with poorer kidney function, as measured by poor glomerular filtration rate, had more brain atrophy, especially subcortical white matter atrophy, and more often white matter lesions and cerebral infarcts. For unrecognized myocardial infarction we found that men, but not women, with unrec-

ognized myocardial infarction had a higher risk of stroke, dementia, white matter lesions, and lacunar infarcts. Thus, these results showed that subclinical vascular disease in the rest of the body is consistently associated with both clinical and subclinical vascular disease in the brain. The question remains, what underlies these associations? One hypothesis is that poor kidney function and ventricular malfunction lead to arterial hemodynamic changes which in turn lead to vascular brain pathology.[37-39] Another possibility is that shared risk factors – such as hypertension, smoking and arteriolosclerosis – explain the interrelationship between kidney disease, heart disease and brain disease.[38] The final common pathway of these mechanisms leading to vascular brain disease could be by affecting the blood supply to the brain.[40] Indeed, in chapter 2.4 we found that total cerebral blood flow as marker of the blood supply to the brain was associated with brain atrophy and that this in turn mediated the association between cerebral blood flow and cognition. However, in chapter 2.5 investigating retinal vessels we found that widening venules rather than narrowing arterioles are related to white matter atrophy. Given that venular widening seems to reflect inflammation and atherosclerosis, whereas arteriolar narrowing is more reflective of hypertension and arteriolosclerosis,[41-43] this points towards other mechanisms also contributing to the relation between cardiovascular risk factors and vascular brain disease. Indeed, there is accumulating evidence that the pathophysiology of cerebral small vessel disease involves not only hemodynamic changes,[12] but also inflammation and atherosclerosis.[44, 45]

Clinical outcomes of structural brain changes

Having studied descriptives and determinants of structural brain changes, we focused on clinical outcomes, especially dementia, depression and mortality (part 3). We were particularly interested in possible differences between white matter and grey matter pathology and the risk of clinical correlates. We further investigated whether we could discern a distinct pattern of atrophy across the various lobes in relation to outcomes.

We found that white matter atrophy was associated with poorer information processing speed and executive function, whereas grey matter atrophy was specifically related with memory performance. Moreover, we found that grey matter atrophy, but not white matter atrophy, was related to the risk of dementia and Alzheimer's disease (chapter 3.1). Previous studies have reported associations of vascular risk factors with cognition, especially information processing speed and executive function, and dementia.[46-49] Our findings provide evidence that vascular factors contribute to cognitive decline and dementia by affecting white matter. In contrast, the pathways underlying the association between grey matter atrophy and memory and dementia seem less vascular driven and possibly involve Alzheimer specific pathology, such as amyloid plaques and neuro-fibrillary tangles.[50, 51]

On a lobar level, atrophy of the hippocampus and temporal grey matter was most strongly associated with dementia, followed by frontal, parietal and occipital grey matter, which corresponds to the pattern found in pathological studies.[52, 53] Interestingly, this pat-

tern remained present even after we excluded persons who had subjective or objective cognitive problems at baseline suggesting temporal differences, in which brain structures are affected already during the pre-clinical phase of the disease process. These temporal differences are further demonstrated when we investigated how many years before clinical onset the various lobes are predictive of dementia, as depicted in Figure 1. Our results emphasize previous findings that dementia has a long pre-clinical phase and that the actual moment of diagnosis does not accurately reflect the disease process.[54]

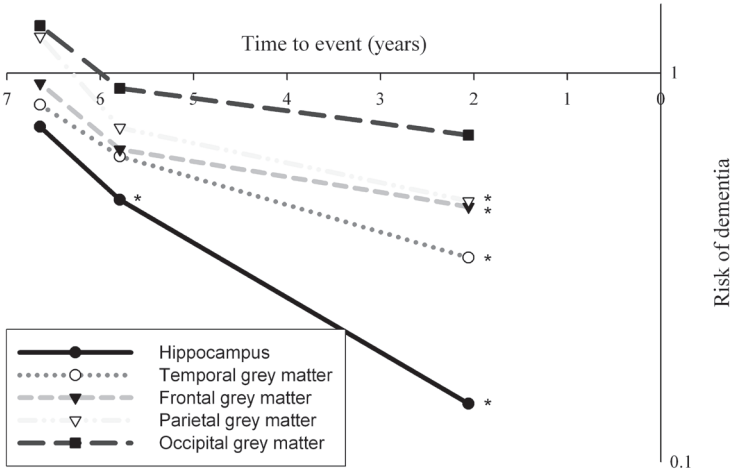

Figure 1. Figure depicting the hazard ratios for dementia against the time to event.
For this analysis, the incident dementia cases from the Rotterdam Scan Study (n=46) were divided into tertiles based on follow-up time till dementia. The non-demented persons were then grouped according to the obtained cut-off values of follow-up time. Hazard ratios were calculated for each tertile using Cox' proportional hazards models adjusted for age and sex.
* denotes significance at $p<0.05$

The vascular depression hypothesis postulates that vascular brain disease is causally related with depression.[55] In line with previous reports,[56-60] we found a strong cross-sectional association between MRI markers of vascular brain disease and depression; markers most strongly associated were parietal and temporal lobe atrophy, deep and frontal white matter lesions, and brain infarcts. However, we found no association between any of the MRI markers and depression prospectively (chapter 3.2). Our results therefore do not support the vascular depression hypothesis, but point towards possible other mechanisms linking vascular brain disease with depression, such as a common etiology,[61] vascular brain disease being related to persistence of depression,[57] or even depression leading to vascular brain disease.[62] This study also showed that repeatedly finding a cross-sectional association should

not automatically become the basis to draw causal inferences unless robust longitudinal data are present.

Finally, we investigated how structural brain changes were related to all-cause mortality and cardiovascular mortality in chapter 3.3. Because brain changes are related with stroke and dementia,[19, 54, 63, 64] we also investigated whether the associations with mortality were independent from these neurological outcomes. We found that brain atrophy, hippocampal atrophy and white matter lesions increased the risk of all-cause mortality. We did not find clear differences between grey matter and white matter, although the risk estimates for white matter atrophy were slightly stronger. The risk of mortality associated with brain atrophy and hippocampal atrophy diminished after censoring persons at onset of dementia. This suggests that it is the dementia process per se, with hippocampal atrophy being a marker thereof, rather than a small hippocampus itself that is associated with mortality. However, it is still largely unknown what are the causes of increased mortality in dementia patients.[65, 66]

Although white matter lesions and lacunar infarcts are predictive of dementia and stroke, the risk of mortality associated with both remained unchanged after censoring for either incident dementia or stroke. Moreover, white matter lesions and lacunar infarcts were particularly related to cardiovascular mortality, suggesting that these MRI markers reflect generalized vascular disease rather than only vascular disease in the brain.

Genetic factors underlying brain changes and neurodegenerative diseases

In the genome-wide association study on stroke described in chapter 4.1 we identified a novel locus that reached genome-wide significance and several highly suggestive loci. The novel locus was consistently associated with stroke across all four discovery cohorts and we were able to replicate the association in an independent sample of different ethnicity. This study also showed the great potential of genome-wide studies when combined in a large internationally organized consortium.

In chapter 4.2 we used data from the Rotterdam Study to confirm the association between *GAB2* and Alzheimer's disease, which was found in a previous genome-wide association study.[67] Consistent with the discovery study, we found that polymorphisms in *GAB2* were related with Alzheimer's disease only in carriers of the *APOE* ε4 allele and not in non-carriers. Meta-analysis of all published results for two polymorphisms showed the same results. Our study is one of the first positive replication of *GAB2* in relation with Alzheimer's disease and opens the way for further studies to investigate the pathways that underlie this relationship.

METHODOLOGICAL CONSIDERATIONS RELATED TO STUDIES IN THIS THESIS

Study design

Both the Rotterdam Study and Rotterdam Scan Study are designed as longitudinal population-based cohort studies, which limits the possibility of selection biases regularly seen in cross-sectional clinic-based samples. Such biases are often due to non-random sampling, case fatality influencing participation, selective attrition leading to loss to follow-up, and competing risks. We sought to tackle these selection biases by taking a random sample from the general population, ensuring a high response rate, using incident cases where possible, and meticulous follow-up of the entire cohort in order to have minimal loss to follow-up. Moreover, all data acquisition, processing, and analyzing was conducted blinded for other parameters that could possibly introduce differential information biases. Finally, because stratification and restriction was not always possible, we used multi-variable modeling to account for confounding biases. Most of the associations we investigated were independent from potential confounding factors. The interpretation of these findings is extensively discussed in the respective chapters.

Imaging studies

The biggest difficulty in interpreting results from neuro-imaging studies is due to differences in data acquisition and phenotype definition between studies. These differences include firstly the fact that each study uses different MR-sequences and classification algorithms, which have been tuned against different golden standards. This introduces considerable inter-study variation and can lead to spurious or blurred effects. For instance, depending on sequences used lacunar infarcts can be classified as white matter lesions or as cerebrospinal fluid and as such contribute to different volumes.[68, 69] We addressed this problem by using a generic classification algorithm that is independent from sequences used.[70]

Secondly, for correction of individual head-size differences, some studies include the posterior fossa in defining intra-cranial volume, whereas others only include supra-tentorial tissues, which leads to considerable volumetric differences of about 15%.[1, 71, 72] Studies focusing on determinants with possible differential effects on the two compartments will therefore be difficult to interpret, if they consider the two compartments together. To elucidate any such confounded associations such studies should also investigate the two compartments separately. In our studies we only investigated the supra-tentorial compartment.

Thirdly, segmentation of lobes is different across studies and usually dependent on landmarks used as boundaries between lobes.[1, 73, 74] This, too, will lead to differences in lobar volumes across studies.

Finally, most neuro-imaging studies, including ours, measure brain MRI markers at only one time point. Even though by expressing volume as percentage of intra-cranial volume we interpreted the data as 'atrophy', real atrophy can only be obtained by measuring at two time points. Thus far, reliable methods for investigating changes in MRI markers in a population-based setting are lacking. However, efforts are underway that could facilitate investigating this in future.[54, 75, 76]

Genetic studies

Most genome-wide studies reported to date have employed a two-stage design: polymorphisms reaching genome-wide significance in a discovery stage or being otherwise highly suggestive are followed up in a replication stage. Usually, the shortlist of polymorphisms for replication is made based on smallest p-values, linkage disequilibrium between the polymorphisms, imputed versus directly genotyped polymorphisms, and possibly known functionality of the polymorphism. However, no clear set of guidelines exists that facilitates similar selection of polymorphisms across various studies.

Moreover, the question remains what is deemed sufficient replication of a locus. Some studies have sought to obtain genome-wide significance for a locus only after joint analysis of the discovery and replication stages;[67, 77] in other studies a locus reaches genome-wide significance already in the discovery stage and then a (Bonferroni corrected) p-value of 0.05 is used for sufficient replication;[78, 79] while even other studies provide data for a locus that reaches genome-wide significance both in the discovery and replication stage separately.[80] An important consideration is that the role of replication is not only to reach statistically significant results (alone or combined with the initial results), but to accumulate evidence that builds on the initial discovery.[81] As such, it is also important to focus on the risk estimates.[81] In the CHARGE consortium we decided a priori not to separate studies into those used for discovery or for replication. We considered that in the meta-analysis each study would serve as independent replication for the other three. A practical reason for this approach is that all participating studies had the logistic and financial infra-structure to obtain genome-wide data. This is in contrast to two-stage genome-wide association studies where only selected polymorphisms need genotyping in the replication phase. An even more important reason is that a meta-analysis of genome-wide data from two studies has more power for identifying novel loci than dividing these studies into one used for discovery and the other for replication.[82, 83] Still, we sought to replicate our findings for stroke in an ethnically different population. We used p=0.05 as threshold for significant replication but also noted that the risk estimates across all studies were strikingly similar.

Another important feature of genome-wide association studies is analyzing large sample sizes in order to have sufficient power to find significant associations. Usually, after the initial (single or two-staged) phase, a second or even a third phase is undertaken with even larger sample sizes to detect loci for which the initial phase was underpowered.[84]

This is often done by simply adding more samples to the initial study population. In a genome-wide association study embedded in a cohort study (e.g the studies participating in the CHARGE consortium), one way to increase the power is to combine prevalent and incident cases. However, if prevalent and incident cases are combined in one logistic analysis, difficulties may arise regarding shared controls, adjustments for covariates, and use of follow-up time. One solution is to run separate analyses for prevalent and incident cases and then combine the results in a meta-analysis. Indeed, the results from a simulation shown in Figure 2 demonstrate that the odds ratios obtained using a logistic regression for the prevalent cases and the hazard ratios using a Cox proportional hazards regression for the incident cases are independent; and that therefore the results can be validly combined using meta-analysis.

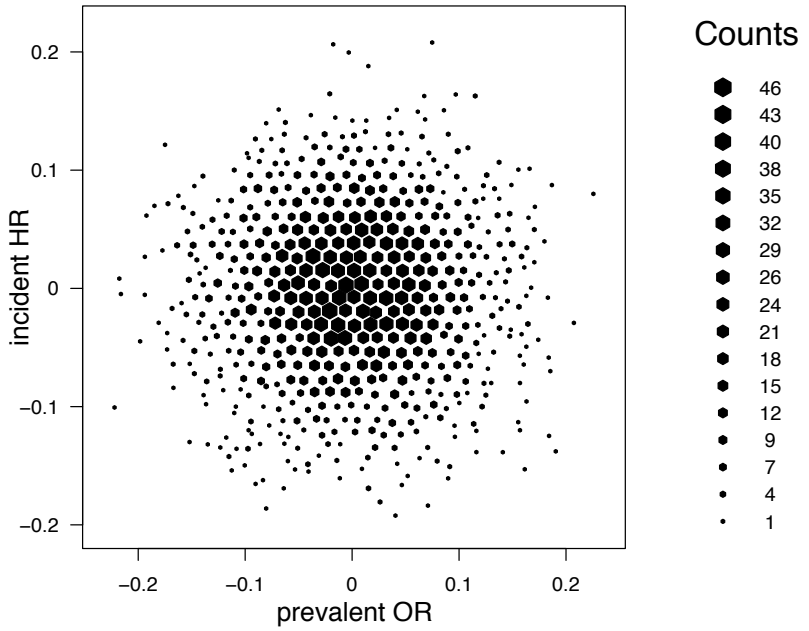

Figure 2. Results of a simulation demonstrating the independence of the odds ratios using prevalent cases and hazard ratios using incident cases from the same study.
In this simulation a cohort of 5000 persons is simulated, of whom 1000 have an event before baseline and we know only their baseline status and 1000 have an even after baseline and we know their follow-up time. The results show that the odds ratios obtained using a logistic regression for the prevalent cases and the hazard ratios using a Cox proportional hazards regression for the incident cases are independent (correlation = -0.008). (Simulation results and figure obtained and reproduced after permission from Dr T. Lumley, University of Washington, USA)

CLINICAL IMPLICATIONS

In order to prevent disease, it is important to first understand its etiology and identify risk factors and risk indicators. Although the studies described may not directly influence clinical practice in the short term, some important points can be made.

First of all, clinicians should be aware of the amount of subclinical pathology prevalent in the general population. This not only relates to the high prevalence of incidental findings on neuro-imaging, but also to the high prevalence of subclinical vascular disease and the strong interrelationship between vascular disease in extracerebral vessel beds and vascular brain disease.

Second, novel modifiable risk factors are of potential interest for research on preventive strategies. Installment of appropriate treatment in men with unrecognized myocardial infarction is one such example. Identification and treatment of subclinical kidney disease may be another. For both examples however, the feasibility of screening and efficacy of any treatment should be investigated first.

A third clinical implication is the use of MRI-markers in the prediction of neurological disease, especially dementia and cognitive impairment. Considerable effort has been put into investigating the predictive value of MRI-markers, in particular hippocampus, for these outcomes.[85, 86] This thesis identifies other MRI-markers that might provide additional discriminative power for detection of persons at high risk of developing dementia. Future research should establish the added value of these MRI-markers, either combined with hippocampal volume or separately.

Finally, all these previous points are readily applicable to the identification of novel genes underlying neurological disease. Novel genes not only give insights into the etiology of disease and identify potential targets for therapies, but can also be used for risk profiling and risk stratification in the prediction of disease.

IMPLICATIONS FOR FUTURE RESEARCH

Neuro-imaging

One of the main and consistent findings of our studies is that white matter atrophy is important in neuro-degeneration, since it relates with vascular brain disease and cardiovascular risk factors and in turn affects clinical outcomes. Future research should focus on disentangling the exact mechanisms underlying these associations. Diffusion tensor imaging (DTI) offers the opportunity to address these issues by investigating the micro-structural integrity of the normal appearing white matter.[87] DTI parameters are considered an earlier marker of neuro-degeneration than structural brain changes. Indeed, initial DTI studies have demonstrated that though white matter atrophy and white matter lesion formation

are closely correlated, they appear to be separate processes;[88] still, more widespread mapping of the normal white matter using DTI is needed to fully comprehend the role of white matter pathology in neuro-degenerative processes. It also remains to be seen whether DTI parameters are related to risk factors and clinical outcomes in a similar way as volumetric parameters of white matter pathology, and if so whether DTI provides information additional to those volumetric measures or whether it is merely a 'proxy' for those.

Although we focused on brain atrophy taking place across the whole brain, several novel focal changes have recently also received considerable interest. Cerebral microbleeds, iron depositions, and Virchow-Rubin spaces are all often present in the aging brain.[89-94] Even though these have been implicated in the etiology of neuro-degenerative diseases, relatively little is known about risk factors and clinical correlates of these focal MRI-markers as compared with white matter lesions and brain infarcts. Further research should consider studying these markers with respect to determinants and outcomes, and the interrelationship with other generalized and focal MRI-markers.

For all our imaging studies, we focused (solely) on the supra-tentorial compartment of the skull. However, based on animal studies it is increasingly being recognized that the cerebellum plays a role in neuro-degenerative diseases.[94,95] Investigating the cerebellum on a population level can provide further important clues regarding the exact function of this thus far underlit structure.

Genetics

The basic premise of genome-wide association studies is that the association is investigated free from any hypotheses. Such studies are therefore by definition hypothesis generating. Once a genome-wide association study has been conducted, the next steps are to replicate the significant and highly suggestive loci in independent samples. Sequencing and functionality studies will contribute in localizing the exact causal variants. Replication not only pertains to the initial phenotype, but also to related (endo)phenotypes. In the genome-wide association analysis presented in this thesis the initial phenotype was stroke and the related endophenotypes would be white matter lesions and lacunar infarcts. Based on the work described in this thesis we might even consider adding white matter atrophy.

In the CHARGE consortium, one of our main strengths was that we used incident stroke cases. However, after considering the potential selection biases and analytical challenges (Figure 2), a next step will be to increase our power by adding the prevalent cases from each study to the meta-analysis. We also have contacts with studies outside CHARGE to perform joint meta-analyses.

Combining genetics and neuro-imaging

Various studies have investigated pre-selected genetic markers underlying MRI markers.[96-98] The advent of genome-wide association studies has meant that instead of a few markers we

Table. The estimated size of a typical dataset of 1000 persons pertaining to different analyses.

		Outcome	
		One aggregated MRI-marker (1 column)	Voxel-by-voxel data (6 million columns)
Determinant	Genotypic data on one polymorphism (1 row)	100Kb	50000Kb
	Genome-wide genotypic data (2.2 million rows)	20000Kb	???? (>1000000Kb)

can now investigate millions of polymorphisms at once, among which we want to detect consistent and robust signals. Currently, efforts are underway to perform genome-wide analyses of MRI markers. In the future, similar to genetic research, technical developments in neuro-imaging will allow investigation of millions of voxel-specific data points instead of aggregated volumetric data that we use now.[99, 100] It is therefore a matter of time before genome-wide association analyses are performed on such voxel-by-voxel MRI data. Although the conceptual background of these analyses will not be very novel, it will pose new challenges with respect to organizing, structuring, analyzing and reporting the large amounts of data. The Table provides rough estimates of filesizes of a single dataset pertaining to different analyses. A typical dataset, in which 2.2 million polymorphisms will be studied against MRI data on a voxel-by-voxel basis (with each voxel being 0.2 mm^3) is estimated to be 1000Mb in size. Current computational infra-structures are underdeveloped to handle such datasets in reasonable time. Moreover, it remains to be seen how to visualize such analyses and whether currently used statistical techniques (e.g. multiple testing corrections) are suited for such data-analysis.

CONCLUDING REMARKS

I have sought to investigate the non-genetic and genetic determinants of structural brain changes and their relationship with neurological outcomes. In this chapter I gave an overview of the results, discussed methodological issues and based on those results gave some recommendations for future research and mentioned clinical implications. It is superfluous to mention that these recommendations and implications are far from exhaustive; however, they do nicely demonstrate the research questions of interest for the coming years, the challenges the investigation of these questions will pose, and the potential breakthroughs the answers for these questions could become.

Indeed, exciting times are ahead!

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Chapter 5.2

Summary

ENGLISH SUMMARY

Neuro-imaging and genetics have played an important role in research on etiologic markers of neuro-degenerative disease. Various neuro-imaging studies have identified focal structural brain changes as in vivo markers of neurodegenerative diseases. It has been suggested that generalized brain changes may also be important markers of neurodegenerative diseases. Only recently have advances in MRI technology and image processing led to the ability to investigate generalized brain changes and their relationship with neurodegenerative diseases. Similarly, the search for genetic markers underlying neurodegenerative diseases has been limited, because genetic research was restricted to single or few preselected markers.

Recently, it has become possible to investigate genetic markers on a large scale across the whole genome.

The aim of the studies described in this thesis was to investigate determinants – both non-genetic and genetic – and clinical outcomes of structural brain changes on MRI. The focus was on markers across the whole brain and whole genome. The studies were embedded within either the Rotterdam Study or the Rotterdam Scan Study, both large prospective population-based cohort studies.

In **Part 2** descriptives and determinants of structural brain changes were described. In **chapter 2.1.1** we quantified brain tissue volumes in people aged 60 to 90 years and investigated how they were related to age, sex, vascular brain disease, and classic cardiovascular risk factors. We found that white matter atrophy seemed to drive loss of brain volume with increasing age. We also found that vascular brain disease and classic cardiovascular risk factors were more associated with white matter atrophy than grey matter atrophy. We concluded that white matter atrophy may be more important in neuro-degeneration than thus far recognized. In **chapter 2.1.2** we report incidental findings in the brain of 2000 participants from the Rotterdam Scan Study. We found that apart from markers of vascular brain changes, the most frequent findings are cerebral aneurysms and benign primary tumors. These occurred in 1.8% and 1.6% of all scans, respectively.

Investigating novel determinants of brain atrophy, we first found in **chapter 2.2** that persons with poorer kidney function had more brain atrophy, especially subcortical white matter atrophy, and more often white matter lesions and cerebral infarcts. Next, in **chapter 2.3** we showed that men, but not women, with unrecognized myocardial infarction had a higher risk of stroke, dementia, and vascular brain disease. Seeking to elucidate the possible mechanism underlying these findings we reported in **chapter 2.4** that total cerebral blood flow – as marker of arterial hemodynamics of the brain – was related with brain atrophy and that this in turn mediated the association between cerebral blood flow and cognition. However, the relation between venular diameter and brain atrophy reported in **chapter 2.5** points to other mechanisms also contributing to the association between vascular risk

factors and vascular brain disease. A common feature between studies in **Part 2** was that subclinical vascular disease in the rest of the body and markers thereof were consistently associated with both clinical and subclinical vascular disease in the brain.

Part 3 of this thesis was dedicated to the relationship of brain changes with clinical outcomes. We started by investigating structural brain changes in relation with cognition, dementia, and Alzheimer's disease in **chapter 3.1** and found that white matter atrophy was associated with poorer executive function and information processing speed, whereas grey matter atrophy was particularly associated with memory performance. Furthermore, we showed that grey matter atrophy, but not white matter atrophy, increased the risk of dementia and Alzheimer's disease. On a lobar level, hippocampal and temporal grey matter atrophy was most strongly associated with dementia, followed by frontal, parietal and occipital grey matter atrophy. Interestingly, this pattern remained present even after exclusion of persons with subjective or objective cognitive problems at baseline. We concluded that atrophy of grey matter and atrophy of white matter have distinct effects on cognition and dementia.

Next, in **chapter 3.2** we investigated the vascular depression hypothesis. Although we found vascular brain disease to be strongly associated with depression cross-sectionally, we could not confirm the vascular depression hypothesis in our longitudinal analyses. In **chapter 3.3** we investigated how structural brain changes were related to all-cause mortality and cardiovascular mortality. We found that structural brain changes on MRI are predictors of mortality, with the associations with brain atrophy and hippocampal atrophy being primarily driven by dementia, whereas those with white matter lesions and lacunar infarcts reflecting generalized vascular disease.

In the genome-wide association study on stroke described in **chapter 4.1** we identified a novel locus that reached genome-wide significance after meta-analysis across four independent studies. The study in **chapter 4.2** was a positive replication of polymorphisms in *GAB2* in relation with Alzheimer's disease. Taken together with previous data, this study suggests *GAB2* as a novel gene modifying the risk of Alzheimer's disease in *APOE*ε4 carriers. Both genetic studies are a starting point to further investigate the pathways that underlie these relationships.

Finally in **Part 5**, I discuss the main findings, methodological limitation, and clinical implications, and I make recommendations for future research. My main conclusions were firstly that white matter atrophy is more important in aging and neuro-degeneration than previously thought. Secondly, subclinical vascular disease in the rest of the body is closely associated with vascular brain disease. Thirdly, grey matter and white matter atrophy are differentially related with cognition, dementia, and to a lesser extent mortality. Finally, genome-wide association studies provide a powerful tool to investigate novel genetic markers underlying neuro-degenerative diseases.

NEDERLANDSE SAMENVATTING

Neuro-imaging en genetica hebben een belangrijke rol gespeeld in het onderzoek naar oorzakelijke markers van neuro-degeneratieve ziekten (zoals beroerte, dementie, ziekte van Alzheimer). Verschillende neuro-imaging studies hebben focale structurele hersenveranderingen geïdentificeerd die als *in vivo* markers dienen van neuro-degeneratieve ziekten. Echter, er is gesuggereerd dat gegeneraliseerde hersenafwijkingen ook belangrijke markers van neuro-degeneratieve ziekten zouden kunnen zijn. Recente ontwikkelingen in MRI technieken en beeldverwerking hebben ertoe geleid dat het nu mogelijk is gegeneraliseerde hersenafwijkingen en de associatie met neuro-degeneratieve ziekten te onderzoeken.

Het onderzoek naar genetische markers van neuro-degeneratieve ziekten was op een vergelijkbare manier gelimiteerd, omdat dat onderzoek zich ook beperkte tot een of enkele voorgeselecteerde genetische markers. Recentelijk is het mogelijk geworden genetische markers over het hele genoom te onderzoeken.

Het doel van dit proefschrift is het onderzoeken van determinanten – zowel niet-genetisch als genetisch – en klinische uitkomsten van hersenveranderingen op MRI. De focus was op markers over het hele brein en het hele genoom. De studies waren ingebed in de Rotterdam Studie (ERGO onderzoek) of de Rotterdam Scan Studie, beide grote prospectieve populatieonderzoeken.

In **deel 2** worden de normatieve waarden en determinanten van hersenveranderingen beschreven. In **hoofdstuk 2.1.1** kwantificeerden wij volumina van de grijze stof, witte stof en cerebrospinale liquor (hersenvocht) in een populatie van 60 tot 90 jaar en onderzochten wij hoe deze gerelateerd waren aan leeftijd, geslacht, vasculaire hersenafwijkingen (wittestoflesies en lacunaire herseninfarcten), en klassieke cardiovasculaire risicofactoren. We vonden dat wittestofatrofie in plaats van grijzestofatrofie ten grondslag ligt aan breinatrofie op hogere leeftijd. Verder vonden we dat vasculaire hersenafwijkingen en klassieke cardiovasculaire risicofactoren met name geassocieerd zijn met atrofie van de witte stof in plaats van de grijze stof. We concludeerden daarom dat wittestofatrofie mogelijk een belangrijkere rol speelt in neuro-degeneratie dan dusver gedacht. In **hoofdstuk 2.1.2** rapporteren we toevalsbevindingen op hersenscans van 2000 deelnemers van de Rotterdam Scan Study. Behalve vasculaire hersenafwijkingen vonden wij dat aneurysmata en benigne hersentumoren de meest voorkomende bevindingen zijn. Deze kwamen respectievelijk in 1.8% en 1.6% van alle scans voor.

Vervolgens onderzochten wij nieuwe determinanten van hersenatrofie. In **hoofdstuk 2.2** vonden we dat mensen met een slechte nierfunctie meer hersenatrofie hebben, met name van de subcorticale witte stof, en vaker wittestoflesies en herseninfarcten hebben. In **hoofdstuk 2.3** tonen we aan dat mannen, maar niet vrouwen, met een niet-herkend hartinfarct een hogere risico lopen op beroerte, dementie, en vasculaire hersenafwijkingen. Om het mogelijk onderliggende mechnisme van deze associaties te onderzoeken rapporteerden we in **hoofdstuk 2.4** dat totale bloedstroom naar de hersenen – als marker van

arteriële hemodynamica in de hersenen – gerelateerd is met hersenatrofie en dat deze associatie vervolgens de mediator is in de relatie tussen totale bloedstroom en cognitie. Echter, de associatie tussen retinale venulaire diameters en hersenatrofie die we rapporteren in **hoofdstuk 2.5** wijst ook naar andere mechanismen die de associatie tussen vasculaire risicofactoren en vasculaire hersenziekten mogelijk verklaren. Concluderend kunnen we zeggen dat het volgende gemeenschappelijk is in alle hoofdstukken van **deel 2**: subklinische vasculaire ziekte buiten het brein en markers daarvan zijn consistent geassocieerd met zowel klinische als subklinische vasculaire ziekten in het brein.

Deel 3 van het proefschrift was gewijd aan de relatie tussen hersenafwijkingen en klinische uitkomsten. Als eerste onderzochten we in **hoofdstuk 3.1** hersenafwijkingen in relatie tot cognitie, dementie en de ziekte van Alzheimer. We vonden dat wittestofatrofie geassocieerd is met slechte scores op cognitieve uitvoeringstaken en snelheidstaken, terwijl grijzestofatrofie met name geassocieerd was met geheugentaken. Verder lieten we zien dat grijzestofatrofie in tegenstelling tot wittestofatrofie het risico verhoogt op dementie en de ziekte van Alzheimer. Toen we de verschillende hersenkwabben apart onderzochten, vonden we dat met name hippocampus atrofie en temporale grijzestofatrofie met dementie geassocieerd zijn, gevolgd door grijzestofatrofie in de frontale kwab, pariëtale kwab, en occipitale kwab. Het was interessant op te merken, dat dit patroon gehandhaafd bleef als we mensen met subjectieve en objectieve cognitieve problemen aan het begin van het onderzoek excludeerden. We concludeerden dat atrofie van de grijze stof en atrofie van de witte stof verschillende effecten hebben op cognitie en dementie.

In **hoofdstuk 3.2** onderzochten we de vasculaire depressie hypothese. Ondanks dat we een sterke associatie cross-sectioneel vonden tussen vasculaire hersenafwijkingen en depressie, konden we de vasculaire depressie hypothese niet bevestigen in onze longitudinale analyses. In **hoofdstuk 3.3** bestudeerden we hoe structurele hersenafwijkingen gerelateerd zijn aan mortaliteit en specifiek cardiovasculaire mortaliteit. We vonden dat structurele hersenafwijkingen op MRI voorspellers zijn van mortaliteit. De associaties van hersenatrofie en hippocampus atrofie met mortaliteit worden gedreven door dementie, terwijl de associaties van wittestoflesies en lacunaire infarcten met mortaliteit een reflectie zijn van gegeneraliseerde vasculaire aandoeningen.

In de genome-wide associatie studie over beroerte beschreven in **hoofdstuk 4.1** hebben wij een nieuw regio geïdentificeerd die genome-wide significant was; voor beide genen in die regio is het plausibel dat zij oorzakelijk gerelateerd zijn aan beroerte. De studie in **hoofdstuk 4.2** is een positieve replicatie van polymorphismes in *GAB2* in relatie met de ziekte van Alzheimer. Samen met voorgaande gepubliceerde data, suggereert deze studie dat *GAB2* een nieuw gen is dat het risico op de ziekte van Alzheimer modificeert afhankelijk van of iemand het *APOE*ε4 allel draagt. Beide genetische studies dienen als startpunt voor verder onderzoek naar de exacte mechanismen van deze associaties.

Ten slotte, in **hoofdstuk 5** bediscussieer ik de belangrijkste bevindingen, de methodologische limitaties, and de klinische implicaties van mijn onderzoek en doe ik suggesties voor verder onderzoek. Mijn belangrijkste conclusies zijn: ten eerste, wittestofatrofie is belangrijker in veroudering en neuro-degeneratie dan tot dusver gedacht. Ten tweede, subklinische vasculaire ziekten buiten het brein zijn sterk geassocieerd met vasculaire ziekte in het brein. Ten derde, grijze stof en witte stof zijn verschillend gerelateerd met cognitie, dementie, en in mindere mate mortaliteit. Ten slotte, genome-wide associatie studies zijn een innovatieve methode om nieuwe genetische markers van neuro-degeneratieve ziekten te identificeren.

Chapter 5.3

Paradoxical Medicine

Sobia Ikram, M. Arfan Ikram, M. Kamran Ikram

Being from a middle class family in Pakistan and studying classic Ayurvedic medicine as a hobby, our maternal grandfather always wanted one of his children to become a medical doctor. For various reasons, mostly financial, this dream never materialised. However, our mother continued to pursue this wish, and eventually three of her children became medical doctors in the Netherlands.

One December the whole family came together to celebrate the holiday season. The weather was cold and wet, and several family members developed a horrible cold and cough.

Being strong proponents of evidence based medicine in our daily clinical practice, we doctors advised various standard remedies such as not going out into cold and dry air too often, stopping smoking, taking cough syrup, etc. Unsurprisingly, none of these helped, so we resorted to advising doing nothing and letting nature take its course.

During our childhood, however, our mother had treated us with herbal medicines to cure common ailments. With time, and influenced by our studies, we became less and less fond of those treatments. Undaunted, our mother now administered such a herbal mixture to those of the family who were coughing. We will never know whether it was the natural course of the illness (no control group), the placebo effect of the mixture (no blinding), motherly love (confounding), or a true healing effect (only a randomised clinical trial could prove this), but everyone's cough disappeared within two to three days.

We would never prescribe this mixture to any of our patients because of lack of clinical evidence, but we can't deny that, after drinking the mixture under strict maternal orders, our coughs, too, started to subside.

Epilogue

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28 F van der Lijn, MW Vernooij, **MA Ikram**, HA Vrooman, D Rueckert, A Hammers, MMB Breteler, WJ Niessen. Automated localization of periventricular and subcortical white matter lesions. In: Pluim JP, Reinhardt JM (editors). Proc. SPIE. Medical Imaging: Image process; 2007, vol. 6512, 651232.

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PHD PORTFOLIO

Name PhD student: MA Ikram **Erasmus MC Department: Epidemiology** **Research School: NIHES**	**PhD period: 1 september 2005 – 1 march 2009** **Promotor(s): MMB Breteler** **Supervisor: -**	
1. PhD training		
	Year	Workload (Hours/ECTS)
General academic skills		
Biomedical English Writing and Communication	2002	1
Research skills		
MSc in Clinical Epidemiology, Nihes, The Netherlands (including courses on methodology, study design, statistical analysis)	2000-2003	30
In-depth courses (e.g. Research school, Medical Training)		
Functionele hersenanatomie en brain imaging relevant voor neuropsychiatrische ziektebeelden, VUMC, The Netherlands	2005	1
Principles of Epidemiologic Data Analysis, Nihes, The Netherlands	2006	1
Conceptual Foundation of Epidemiologic Study Design, Nihes, The Netherlands	2006	1
Bayesian Statistics, Nihes, The Netherlands	2006	0.9
Spatial Statistics, Nihes, The Netherlands	2006	0.9
Training course in Genome Wide Association Studies, Dept Epidemiology, Erasmus MC, The Netherlands	2007	1
Good clinical practice, Erasmus MC, The Netherlands	2007	1
R-course, Erasmus MC, The Netherlands	2008	1
The Genetics of Complex Disorder, Broad Institute, Boston, MA, USA	2008	1
(Inter)national conferences – participation and presentations		
Pre-conference Imaging Consortium, Madrid, Spain. Oral: *Brain tissue volumes in the elderly*.	2006	1.3
International Conference on Alzheimer's Disease and Related Disorders, Madrid, Spain. Poster: *Brain tissue volumes in the elderly*.	2006	1.3
59th Annual Meeting of the American Academy of Neurology, Boston, MA, USA. Poster: *Brain tissue volumes in relation to cognitive function and dementia*.	2007	1.3
Research Institute for Diseases in the Elderly symposium, Amsterdam, The Netherlands. Oral: *MRI and neurodegenerative diseases*.	2007	0.3
14th Nordic Meeting on Cerebrovascular diseases, Arhus, Denmark. Keynote speaker: *White matter lesions - silent, but not harmless*.	2007	1.7

60th Annual Meeting of the American Academy of Neurology, Chicago, IL, USA. Posters: *Unrecognized myocardial infarction increases the risk of stroke, dementia and cerebral small vessel disease* and *Kidney function is related to cerebral small vessel disease*.	2008	1.6
Consortium meeting 'Cerebral Mircobleeds: Detection and Definition'. Chicago, IL, USA	2008	0.5
Consortium meeting 'CHARGE – Neuro-working group'. Boston, MA, USA	2008	0.5
Consortium meeting 'CHARGE – General meeting'. Seattle, WA, USA. Oral: *Meta-analysis of genome-wide association studies on stroke in CHARGE*.	2008	1.3
Consortium meeting 'CHARGE – Neuro-working group'. Boston, MA, USA	2008	0.5
Vas-Cog conference 2009, Singapore. Oral: *The role of retinal vascular caliber in brain atrophy on MRI* and *White matter microstructural integrity and cognitive function in a general elderly population*. Poster: *Structural markers of vascular brain disease in relation to depression in the elderly*	2009	2

Other		
Co-worker for the national registry and expertise center on Creutzfeldt-Jacob's disease, Erasmus MC, The Netherlands	2006-2009	7

2. Teaching activities

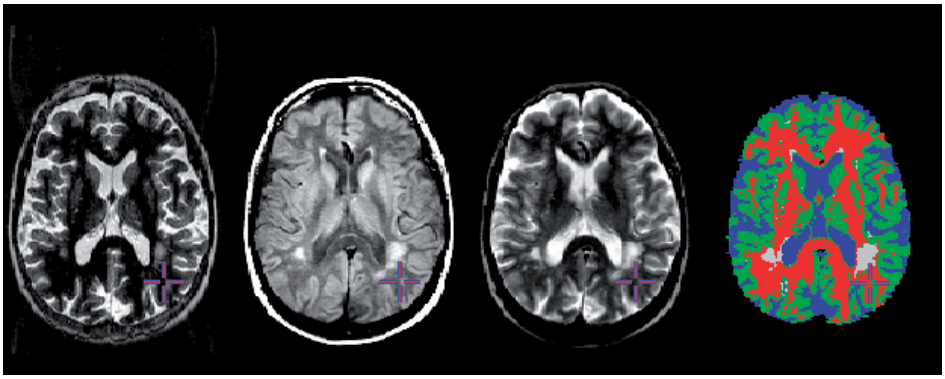
	Year	Workload (Hours/ECTS)
Lecturing		
Teaching assistant for course 'Principles of Epidemiology' in the Erasmus Summer Programme	2006/2007	2
Supervising practicals and excursions		
Supervising practicals on statistics	2006	0.5
Supervising practicals on epidemiology	2006/2007	1
Supervising Masters thesis		
Supervised Mariëlle Poels: Total cerebral blood flow in relation to cognitive function: The Rotterdam Scan Study	2007	6
Supervised Millad Solouki: Unrecognized myocardial infarction and the risk of mortality. The Rotterdam Study	2007	6
Member of MSc committee of Balinder Paul: Segmentation of the structure of hippocampus in MRI neuroimages	2008	0.5

CURRICULUM VITAE

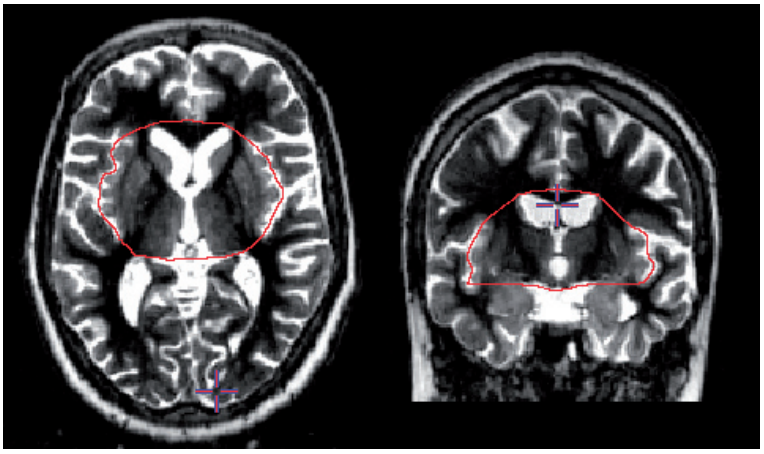
Mohammad Arfan Ikram was born on December 20th 1980 in Rotterdam, The Netherlands. After graduating in 1999 at the Erasmiaans Gymnasium, he started with his medical studies at the Erasmus University Rotterdam. During the second year, he was invited to participate in the Master of Science in Clinical Epidemiology program by the Netherlands Institute of Health Sciences. During this program he received his initial training in epidemiology, part of which was spent at the Harvard School of Public Health in Boston, USA during the Tenth Annual Summer Session. In 2003 he obtained both his MSc degree as well as his doctoral medical degree. After two years of internships he completed his medical studies in 2005. Subsequently, he started the work described in this thesis under the supervision of Prof.dr. Monique Breteler. Upon defending his thesis, he will continue working at the department of Epidemiology. From July 2009 onwards, he will then start his residency in Radiology (head: Prof.dr. GP Krestin) at the Erasmus MC.

Appendix

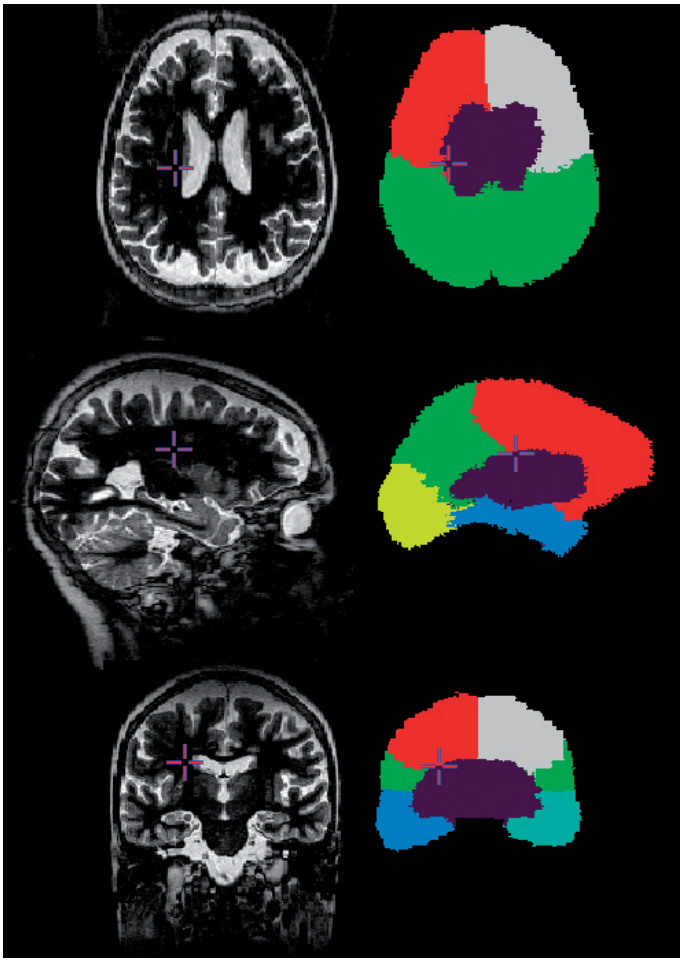
COLOR FIGURES



Chapter 2.1.1 Figure 1. From left to right: HASTE-Odd scan, Proton-density weighted scan, T2 weighted scan, and result after tissue classification and removal of non-brain tissues (no manual reclassification performed; blue: cerebrospinal fluid; green: grey matter; red: white matter; white: white matter lesion).



Chapter 2.2 Figure 1. HASTE-Odd sequence, in which the boundary (red line) between the deep and lobar brain regions is delineated, according to the protocol by Bokde et al.[29, 30]



Chapter 3.1 Figure 1. Haste-Odd sequence and the result after segmentation into the various brain lobes using non-rigid registration. Red: left frontal lobe; white: right frontal lobe; dark green: left parietal lobe; light green: right parietal lobe; dark blue: left temporal lobe; light blue: right temporal lobe; yellow: left occipital lobe; purple: central region comprising basal ganglia and corpus callosum. The right occipital lobe is not shown on these cross-sections.