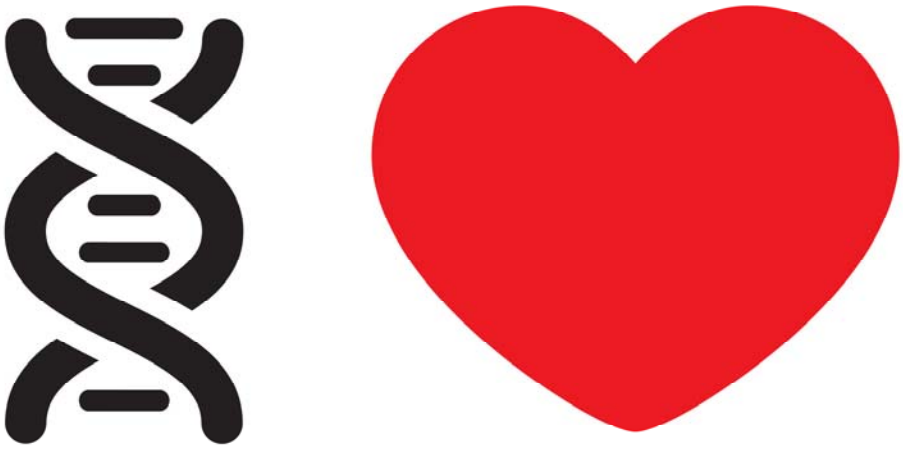

Determinants of Subclinical Vascular Brain Disease in Aging

Benjamin F. J. Verhaaren

Determinants of

Subclinical Vascular Brain Disease in Aging

Benjamin F.J. Verhaaren

Acknowledgements

The work presented in this thesis was conducted at the department of Epidemiology and the department of Radiology of the Erasmus MC, Rotterdam, the Netherlands.

The Rotterdam Study is supported by the Erasmus MC and Erasmus University Rotterdam, the Netherlands Organization for Scientific Research (NWO), the Netherlands Organization for Health Research and Development (ZonMW), the Re-search Institute for Diseases in the Elderly (RIDE), the Netherlands Genomics Initiative, the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII) and the Municipality of Rotterdam.

The contributions of the inhabitants, general practitioners and pharmacists of the Ommoord district to the Rotterdam Study are gratefully acknowledged.

Financial support for the publication of this thesis was kindly provided by the Departments of Epidemiology and Radiology of the Erasmus MC, and the Dutch Heart Foundation (Nederlandse Hartstichting), grant number: 2009B102.

ISBN: 978-90-5335-827-6
Lay-out: Ridderprint BV, Ridderkerk, the Netherlands; cover idea B.F.J. Verhaaren
Print: Ridderprint BV, Ridderkerk, the Netherlands

Determinants of Subclinical Vascular Brain Disease in Aging

Determinanten van
Subklinische Vasculaire Hersenziekte bij Veroudering

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 16 april om **11.30 uur**

door

Benjamin Frederik Johannes Verhaaren

geboren te Great Yarmouth (Groot-Brittannië)

ERASMUS UNIVERSITEIT ROTTERDAM

PROMOTIECOMMISSIE

Promotoren:

Prof.dr. A. Hofman

Prof.dr. A. van der Lugt

Overige leden:

Prof.dr. C.M. van Duijn

Prof.dr. G.J. Biessels

Dr. M.J. O'Sullivan

Copromoteren:

Dr. M.A. Ikram

Dr. M.W. Vernooij

funded by the dutch heart foundation

Hartstichting

The research described in this thesis was supported by a grant of the Dutch Heart Foundation (project number 2009B102*)*. Financial support by the Dutch Heart Foundation for the publication of this thesis is gratefully acknowledged.

CONTENTS

Chapter 1

General Introduction

We are at the dawn of a large epidemic of neurodegenerative disease and cerebrovascular disease that will have major impact on our society. It is estimated that currently at least 35 million people live with dementia worldwide and this number is expected to double every twenty years: 66 million in 2030 and 115 million in 2050.[1] At the same time, every year 15 million people suffer from a stroke.[2] Apart from imposing a huge burden on individuals and families, no other disease costs the society more than these.[3] It is not surprising that governments of countries have recently started to invest billions of dollars and Euros in brain research.

But where to start tackling this epidemic? We may already be too late at the time of diagnosis, as in both dementia and stroke irreversible brain damage is already present, for which there is at present no treatment available. Advances in research have shown that for both diseases, signs of disease may already be detectable in the brain years and even decades in advance.[4] These subtle signs generally go unnoticed and therefore are often called *subclinical brain disease*. In particular vascular pathology, such as the presence of white matter lesions, has been shown to already manifest in the middle-aged and was found to increase the risk of both dementia and stroke. Subclinical brain disease can be visualized with magnetic resonance imaging (MRI) in vivo and has shown to be highly prevalent in the aging population. Yet, its exact etiology remains to be elucidated. Studying these imaging markers and their determinants may help us understand their etiology better and aid in identifying potential modifying factors or pathways for intervention. Population-based cohort studies lend themselves well for this purpose as people without disease can be followed for years with imaging, cognitive testing and the assessment of possible determinants, including genetic factors. Although genes themselves would be difficult to modify, they can point to modifiable pathophysiological pathways of stroke and dementia.

In recent years, developments in genetics and imaging have revolutionized the field. Genome-wide association studies have emerged in which the relation between millions of DNA variants and traits, such as a disease, can be studied. Developments in the field of imaging have made it possible to automatically quantify brain pathology non-invasively in vivo. Furthermore, novel imaging methods have been developed to detect subtle structural brain changes which were not visible before to the human eye. An example is diffusion tensor imaging in which the microstructural integrity of brain tissue can be studied non-invasively. These developments in genetics and imaging have greatly increased the potential to identify novel determinants of neurodegenerative disease and cerebrovascular disease and to investigate their role in subclinical brain disease in the general population.
For this thesis I therefore investigated determinants of subclinical brain disease within the Rotterdam Study, a large population-based study in the Netherlands, in which both genome-

wide data and neuro-imaging data are acquired.[5] Furthermore, to increase the ability to find novel determinants we collaborated with other population-based studies in France, Germany, Great Britain, Iceland, Singapore, and the United States. For this thesis I mainly focused on subclinical *vascular* brain disease.

In chapter 2, I discuss the projects related to the development of new methodology to analyze brain imaging data. Chapter 2.1 describes a novel method to quantify white matter lesions in multiple locations in the brain. Such a method facilitates the study of whether lesions in different areas have different causes and consequences. Chapter 2.2 describes the application of diffusion tensor imaging to quantify subtle white matter abnormalities on MRI. With this method we investigated whether loss of the microstructural integrity of the white matter is present before visible lesions develop.
In chapter 3 of this thesis I describe my work on the genetic determinants of subclinical brain disease. In chapter 3.1 I present the results from our study in which we sought to replicate a recently found genetic locus for white matter lesions in an independent sample. Such efforts contribute to the generalizability and validity of genetic loci that are identified in genome-wide association studies. Chapter 3.2 describes our genome-wide approach to identify additional loci for white matter lesions in a collaboration with imaging studies from France, Germany, Great Britain, Iceland, Singapore, and the United States. In chapter 3.3, I investigated whether recently discovered Alzheimer's disease genes already affect cognition in non-demented people and whether they may improve the prediction of dementia. In chapter 3.4, I present our research on the relation between the genetics of blood pressure and the retinal and cerebral circulation. Such research adds to our understanding of how blood pressure influences the perfusion of the retina and the brain.
Chapter 4 of my thesis is on non-genetic determinants of subclinical brain pathology. In chapter 4.1 we compare the incidence of dementia and the presence of brain pathology on MRI in a cohort from 1990 with a cohort from 2000. Such comparisons are important for policy makers to pick up trends in dementia and brain pathology over time. In chapter 4.2 I touch upon the relation between uric acid and brain atrophy and cognition. In chapter 4.3 I discuss our study of the relationship between blood pressure and the progression of white matter lesions.
Finally, in chapter 5 I conclude with a review of my main findings and discuss implications and suggestions for future research.

REFERENCES

1. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimer's & dementia : the journal of the Alzheimer's Association.* Jan 2013;9(1):63-75 e62.
2. Cumming T, Brodtmann A. Dementia and stroke: the present and future epidemic. *International journal of stroke : official journal of the International Stroke Society.* Dec 2010;5(6):453-454.
3. Luengo-Fernandez R, Leal J, Gray AM. UK research expenditure on dementia, heart disease, stroke and cancer: are levels of spending related to disease burden? *European journal of neurology : the official journal of the European Federation of Neurological Societies.* Jan 2012;19(1):149-154.
4. Ikram MA, Vrooman HA, Vernooij MW, et al. Brain tissue volumes in the general elderly population. The Rotterdam Scan Study. *Neurobiology of aging.* Jun 2008;29(6):882-890.
5. Hofman A, van Duijn CM, Franco OH, et al. The Rotterdam Study: 2012 objectives and design update. *Eur J Epidemiol.* Aug 30 2011.

Chapter 2

Methodological Topics

Chapter 2.1

Automated measurement of local white matter lesion volume

Fedde van der Lijn, Benjamin F.J. Verhaaren, M. Arfan Ikram, Stefan Klein,
Marleen de Bruijne, Henri A. Vrooman, Meike W. Vernooij, Alexander Hammers,
Daniel Rueckert, Aad van der Lugt, Monique M.B. Breteler, Wiro J. Niessen.

Neuroimage. 2012 Feb 15;59(4):3901-8.

ABSTRACT

It has been hypothesized that white matter lesions at different locations may have different etiology and clinical consequences. Several approaches for the quantification of local white matter lesion load have been proposed in the literature, most of which rely on a distinction between lesions in a periventricular region close to the ventricles and a subcortical zone further away. In this work we present a novel automated method for local white matter lesion volume quantification in magnetic resonance images. The method segments and measures the white matter lesion volume in 43 regions defined by orientation and distance to the ventricles, which allows a more spatially detailed study of lesion load. The potential of the method was demonstrated by analyzing the effect of blood pressure on the regional white matter lesion volume in 490 elderly subjects taken from a longitudinal population study. The method was also compared to two commonly used techniques to assess the periventricular and subcortical lesion load. The main finding was that high blood pressure was primarily associated with lesion load in the vascular watershed area that forms the border between the periventricular and subcortical regions. It explains the associations found for both the periventricular and subcortical load computed for the same data, and that were reported in the literature. But the proposed method can localize the region of association with greater precision than techniques that distinguish between periventricular and subcortical lesions only.

INTRODUCTION

Elderly individuals often exhibit hyperintense lesions on T2-weighted or fluid attenuation inversion recovery (FLAIR) magnetic resonance images (MRI) of the brain. There has been extensive debate whether these white matter lesions (WMLs) have a different underlying etiology, or whether clinical consequences vary depending on their location in the brain. For example, WMLs located in the periventricular areas (PVWMLs) were related to cognitive decline, whereas WMLs found in the subcortical white matter (SCWMLs) were associated with depression.[1,2] A three year follow-up MRI study showed a larger rate of progression of subcortical WMLs compared to periventricular WMLs.[3] Other authors have argued that a distinction between PVWMLs and SCWMLs may not be biologically plausible or clinically relevant. Some pathological studies point to a common ischemic etiology of severe PVWML and SCWMLs (see DeCarli et al.[4] and Gouw et al.[5] for an overview). However, the pathological substrate of WMLs visible on MRI has been found to be quite heterogeneous.[5] Furthermore, DeCarli et al.[4] showed that PVWML and SCWML load are highly correlated with the total WML load and with each other. Traditionally, analyses of local WML severity are based on scoring the lesion loads in the periventricular and subcortical areas. The most commonly used measurements of PVWMLs and SCWMLs are visual rating scales that have separate scores for the two WML.[2,6,7] A number of more recent studies describe automated PVWML/ SCWML analyses.[4,8,9] These methods all follow a similar strategy: WML voxels obtained from an automated lesion segmentation are labeled as periventricular if they are located within a user-defined distance from the ventricular wall. The boundary between the periventricular and subcortical zones is usually positioned in the vascular watershed area, between 3 and 13 mm from the ventricles.

A second approach to local WML analysis is regional volume measurement. This type of method provides more detailed spatial information by determining the regional WML load, e.g. in the cerebral lobes,[8,10] or by creating lesion probability maps that show the lesion frequency in a study population per voxel location in a standardized coordinate system.[4,8,11,12] Regional methods involve a trade-off between spatial resolution and power to detect associations between clinical variables and WMLs further away from the ventricles. At these locations the probability of encountering a lesion is much smaller, because WMLs tend to spread over a larger area.[12] This reduces the sensitivity of methods based on lesion probability maps to detect an association in the subcortical region. Aggregating over the entire subcortical region solves this problem, but it reduces the spatial resolution of the analysis.

In this work, we present an automated method for regional WML measurement that is somewhere between the global PVWML/ SCWML distinction and a voxel-based lesion probability map. It measures WML volume, obtained with an automated segmentation

technique, in 43 regions defined by their distance and orientation with respect to the ventricles. The potential of the method is demonstrated by analyzing the relation between blood pressure and regional WML volume for 490 elderly subjects taken from a population-based imaging study. The results are compared with a visual rating of the PVWML/SCWML load and an automated measurement that measures the PVWML/SCWML volume based on a ventricle segmentation.

MATERIALS AND METHODS

Subjects

The study population was derived from the Rotterdam Study, a large population-based cohort study in the Netherlands that started in 1990 and investigates the prevalence, incidence, and determinants of chronic diseases in the elderly.[13,14] From 1995 to 1996 we randomly selected 965 participants of the Rotterdam Study between 60 and 90 years of age to participate in the Rotterdam Scan Study to investigate age-related brain abnormalities on MRI.[15] After excluding persons who were demented or had MRI contraindications, 832 participants were invited. Among these, 563 persons gave their written informed consent and participated in the study (response rate 68%). Of this group, 52 persons developed claustrophobia during MRI acquisition. Twenty one datasets were unusable for analysis, leaving a total of 490 participants with complete and usable MRI data.[16,17] The study protocol was approved by the medical ethics committee of the Erasmus MC, Rotterdam, The Netherlands.

Image data

The image datasets were acquired with a 1.5 T Siemens Vision MR unit. The protocol included three axial sequences, T1-, T2-, and proton-density-weighted scans (PDw, T1w and T2w), as well as a custom-made 3D Half-Fourier Acquisition Single-Shot Turbo Spin Echo (HASTE) sequence that was acquired in the coronal direction.

The T1w was obtained with the following parameters: TR 700 ms, TE 14 ms, NEX 1, matrix size 192×256, flip angle 70°, slice thickness 5.0 mm, inter-slice gap 1 mm. The T2w was acquired with TR 2200 ms, TE 80 ms, NEX 1, matrix size 192×256, flip angle 80°, slice thickness 5.0 mm, inter-slice gap 1 mm. The PDw was acquired with TR 2200 ms, TE 20 ms, NEX 1, matrix size 192×256, flip angle 80°, slice thickness 5.0 mm, inter-slice gap 1 mm. These axial scans were each reconstructed to 20 slices (256×256) with a voxel dimension of 1.0×1.0×5.0 mm. The HASTE was made with: TI 4400 ms, TR 2800 ms, matrix size 192×256, flip angle 180°, slice thickness 1.25 mm. Two HASTE modules were sequentially acquired after the inversion pulse of which we used the one with an effective TE of 29 ms. The HASTE module combined

non-selective radio frequency excitations to provide a short inter-echo spacing of 3.9 ms. The inversion time was chosen to eliminate the signal from the white matter and the resulting contrast is equivalent to an inverted T1-weighted gradient echo scan. The HASTE image was reconstructed to 128 slices (256×256), with a voxel dimension of 1.0×1.0×1.25 mm.
All scans were corrected for MR bias fields with N3.[16] Non-rigid registration of a manually labeled mask (excluding the posterior fossa) was used to measure the intracranial volume (ICV).[17]

Blood pressure measurement

The participants' blood pressure was measured on the right upper arm with a random-zero sphygmomanometer, and the average of both was used. The use of blood-pressure-lowering medication was assessed during an interview. One subject was excluded from the analysis, because the blood pressure measurement was not available.

Regional WML volume measurement

To investigate the role of lesion location we developed a novel method that quantifies WML volume in 43 regions defined by their distance and orientation with respect to the ventricles. The technique consists of four steps: lesion segmentation, ventricle segmentation, region segmentation, and regional WML volume measurement.
First, all images were segmented in background (BG), gray matter (GM), white matter (WM), cerebrospinal fluid (CSF), and WMLs with a supervised voxel classification method.[18] This technique used a k-nearest-neighbor classifier to label all voxels based on their intensities on the T2w, PDw, and HASTE images. For this purpose the T2w and PDw scans were first co-registered to the HASTE and resampled to the HASTE resolution. The method was trained on 12 manually labeled datasets. The final labeling was obtained by considering 45 neighbors in the three-dimensional feature space spanned by the MR intensities. The accuracy of this method was tested with leave-one-out experiments on the 12 manually segmented datasets.[17] The average WML Dice similarity index was 0.63 and the volumetric ICC was 0.84.
As a second step we segmented the ventricles in the HASTE images, by non-rigidly registering 20 atlas images to the target images using the Elastix registration software.[19] The registration consisted of an affine step followed by a non-rigid transformation parameterized with cubic b-splines. We used mutual information as a similarity measure. Both registration steps were performed in a multi-resolution scheme. For every resolution the b-spline control point spacing was decreased with a factor of two, up to a final distance of 10 mm in all directions. The optimizations were performed using a stochastic gradient descent.
The atlas images contained 83 manually labeled brain areas, including the ventricles.[20] By applying the deformation obtained with the registration to the ventricle regions and averaging the results, a ventricular probability map in the coordinate system of the target image

was constructed. We then used this probability map as input for an automated brain structure segmentation method.[21] This method also used a CSF probability map that was obtained with the k-nearest neighbor classifier described above. To obtain a ventricle segmentation, the ventricular and CSF probability maps were combined with a Markov random field regularizer in an energy model that was globally optimized using graph cuts.

In the third step, 43 regions were defined by dividing the brain in three sections that contain the three periventricular locations distinguished in the visual rating described below: the regions adjacent to the frontal horns, to the occipital horns, and to the main body of the lateral ventricles. We did not choose a lobar subdivision because not all white matter surrounding the occipital horns belongs to the occipital lobe; some of this area is part of the parietal and frontal lobes. The same posterior WML will therefore contribute to three different lobar volume measurements, which would complicate the interpretation.

The three sections were based on the 83 atlas regions described above. We deformed the regions to the target coordinate system and combined the results with a vote rule.[22] The 83 regions were then merged to form the anterior, posterior, and central section. The anterior section covered the frontal lobe excluding the precentral gyrus. The posterior section contained the entire occipital lobe, a large part of the parietal lobe, and the precentral gyrus. The central section consisted of the area around the basal ganglia up to and including the insula, as well as the corpus callosum and the cingulate gyrus. The anterior part of the temporal lobe up to and including the hippocampus, the cerebellum and the brain stem, were excluded from the analysis as they contained very few WMLs. An exact list of the composition of the three sections, as well as the excluded regions can be found in Appendix A.

These three sections were then further subdivided in 15 equidistant shells around the segmented ventricles. The first boundary was located at 2 mm from the ventricles, and the

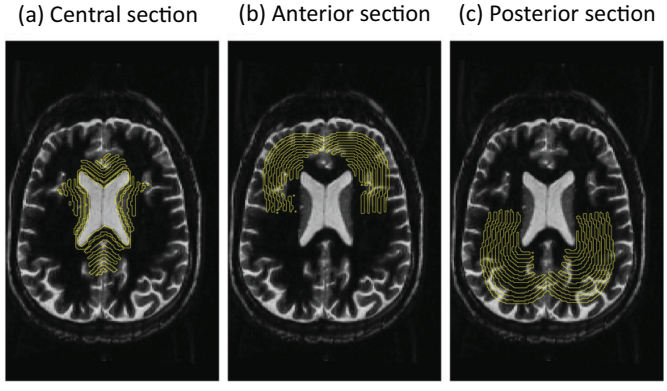

Figure 1. HASTE image of the brain with a subdivision in 43 regions shown for the central (left panel), anterior (middle panel) and posterior (right panel) sections. For the purpose of illustration every other boundary is not shown in the figure.

last one at 28 mm. Lesions more than 28 mm from the ventricles were considered part of the last shell. Most of the WML voxels directly adjacent to the posterior and anterior horns of the ventricles fell into the corpus callosum or caudate nucleus regions, which are part of the first central shell. As a result it was not possible to accurately estimate the lesion volume in the first shell of the anterior and posterior sections. We therefore considered all WML voxels up to 2 mm as part of the first central shell.
This scheme resulted in a subdivision of the brain in 15×3-2=43 regions (Figure 1). In the last step, the volumes of the segmented WMLs were measured for each region.

Visual periventricular and subcortical WML rating

The PVWML and SCWML load was also assessed visually for all subjects by two experienced raters out of a pool of four, using the T1w, T2w and PDw images printed on hard copy with a reduction factor of 2.7 compared to the original scan resolution. The PVWML load was rated separately for regions adjacent to the anterior horn, occipital horns, and the main body of the ventricles on a scale of zero to three. A score of zero represented no lesions, one a pencil-thin lining, two a smooth halo, and three large confluent lesions. The three regional ratings were then summed to obtain a score between zero and nine. Subcortical lesions were counted and divided in three size categories based on their largest diameter. The total SCWML load was estimated by assigning a fixed volume of 0.004, 0.1, and 0.9 ml for every lesion in each category respectively.[2] In the latter reference, intra- and interrater weighted kappas for the PVWML grade of 0.79 and 0.90 were reported. The SCWML inter- and intra-rater intra-class correlation coefficients (ICCs) had been 0.88 and 0.95.[2]

Automated periventricular and subcortical WML measurement

Finally, PVWML and SCWML volumes were measured with an automated method. For this measurement we divided the brain into two regions based on distance to the segmented ventricles. The periventricular and subcortical regions were obtained by computing a Euclidean distance transform from the ventricle segmentation. All segmented WML voxels within a distance of 7 mm were labeled as PVWML, the rest was labeled as SCWML.

Data analysis

The spatial distribution of the WMLs was analyzed by plotting the first quintile, median, and fourth quintile of the lesion volume per section and per region. The relation between blood pressure and regional WML volume was investigated with multiple linear regression. These analyses were adjusted for age, sex, ICV, and the use of bloodpressure-lowering medication (coded as binary variable). To make the regression coefficients comparable between the regions, we converted all regional WML volumes to Z-scores by subtracting the population median and dividing by the interquartile range.

Since the regional WML volume is low for most subjects and always positive, the residuals of the multivariate linear model are not normally distributed. We therefore used a randomization test with 10000 permutations under a reduced model to compute p-values for the null-hypothesis that the slope is.[23,24] The randomization test was also used to correct for multiple comparisons. This was done by comparing the t-value associated with the slope of each regional regression to the null distribution of maximum t-values computed over all regions.[25]

To compare the regional distribution of WMLs to the periventricular and subcortical lesion loads, first quintiles, medians, and fourth quintiles were computed for the visual and automated PVWML/ SCWML scores. We then computed the association between blood pressure and the risk of severe PVWML and SCWML load with multiple logistic regression for both the visual and automated scores. Severe WMLs were defined as the upper quintile of the score or volume distribution. This definition is similar to the one used in a previously published analysis of the relation between blood pressure and WML load in the Rotterdam Scan Study.[26] Since the sample analyzed in this work is a subset of their study population, the results should be comparable. Analyzing the risk of severe lesions also allows us to compare effect sizes for the discrete visual PVWML/ SCWML ratings, and the automated PVWML/ SCWML volume measurements. All analyses were adjusted for age, sex, ICV, and the use of blood-pressure-lowering medication.

RESULTS

Subjects

The characteristics of the study population are shown in Table 1.

Table 1. Characteristics of the study sample.

	N=489
Age at time of scan, years	73.4 (7.9)
Female sex, N(%)	248 (50.7)
Systolic blood pressure, mmHg	145.8 (20.6)
Diastolic blood pressure, mmHg	76.6 (11.5)
Use of blood-pressure-lowering drugs, N (%)	185 (37.8)
Intracranial volume, ml	1128 (116)

Values are means (standard deviation) unless specified otherwise.

Regional WML analysis

The first quintile, median, and fourth quintile of the WML volume per section were: 1.2, 3.6, and 8.2 ml for central; 0.6, 1.4, and 3.9 ml for anterior; and 0.9, 3.2, and 10.6 ml for posterior.

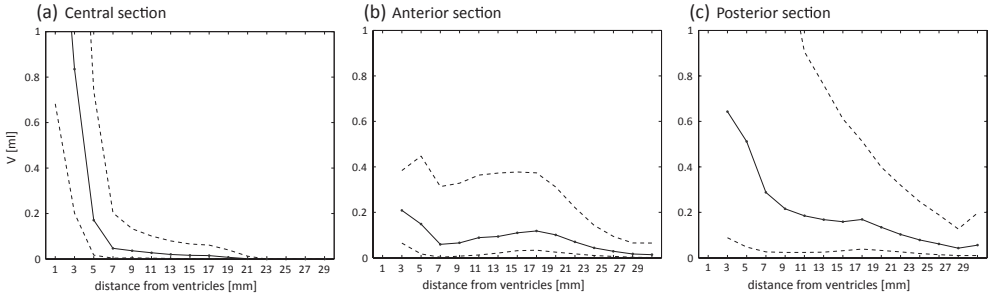

Figure 2. WML volumes in the consecutive shells with increasing distance to the ventricles in the central, anterior, and posterior sections. The solid line marks the median, the dashed lines the first and fourth quintile.

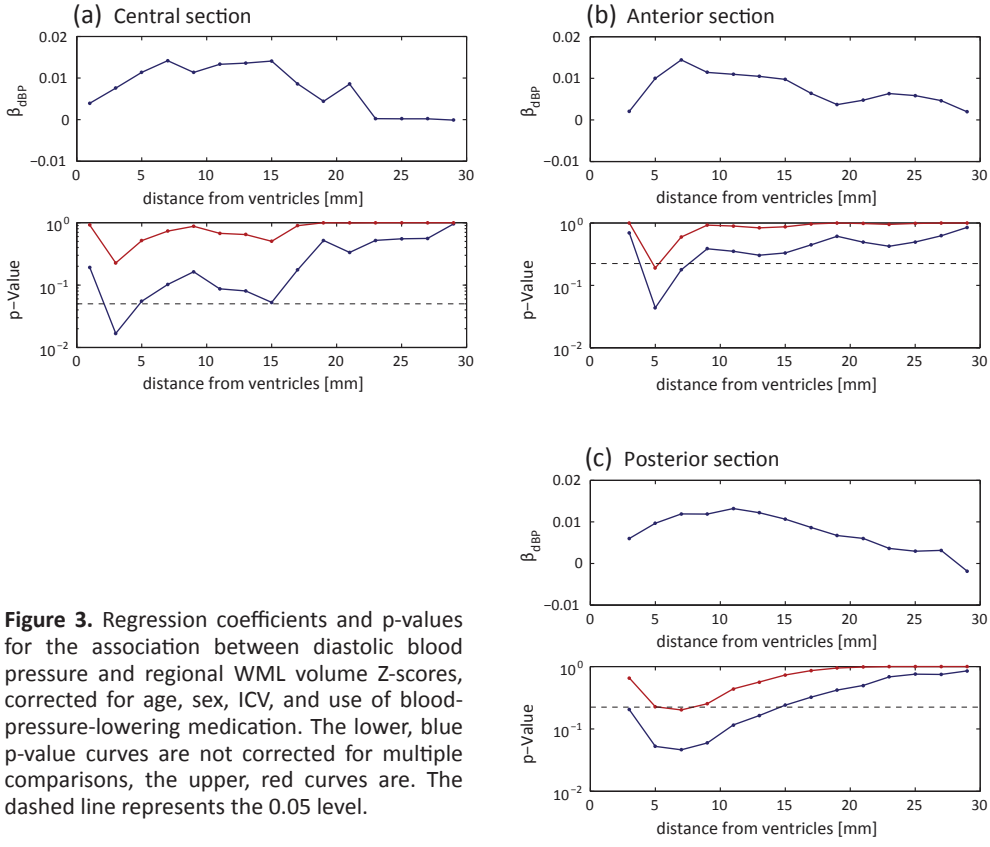

Figure 3. Regression coefficients and p-values for the association between diastolic blood pressure and regional WML volume Z-scores, corrected for age, sex, ICV, and use of blood-pressure-lowering medication. The lower, blue p-value curves are not corrected for multiple comparisons, the upper, red curves are. The dashed line represents the 0.05 level.

For the excluded brain regions these scores were 0.00, 0.02, and 0.07 ml. Figure 2 shows the spatial distribution of the WMLs per section. The central section had the highest volumes in the first shell, which includes all lesion voxels directly adjacent to the ventricles. Moving

away from the ventricles the lesion load quickly dropped. The posterior and anterior section can be divided in three parts. The first part starts at 2 mm with the largest regional WML load, which then decreases until about 10 mm. Moving further away from the ventricles, the regional load stabilizes in the posterior section and rises slightly in the anterior section. After 18 mm from the ventricles the regional load starts to decrease again until it reaches negligible quantities.

Figure 3 shows the strength of the relation between diastolic blood pressure and normalized regional WML volume, as a function of distance to the ventricles. The three sections show roughly the same pattern: the association strengths reach a maximum after the first few shells and then slowly drop as the distance to the ventricle increases. p-Values in the first shell are relatively large, and then drop to a minimum in the second shell. Moving away from the ventricles, the values start rising again.

Figs. 4a and b show the anterior and posterior shells for which the relation between diastolic blood pressure and regional WML volume was borderline statistically significant after correcting for multiple comparisons. Figs. 5a and b show the fit of the blood pressure model for two of these regions. None of the regions in the central section had a statistically significant relation between diastolic blood pressure and lesion load.

We did not find any statistically significant relation between systolic blood pressure and WML volume. The regional association strength curves were relatively flat (data not shown).

Periventricular and subcortical WML analysis

The first quintile, median, and fourth quintile of the visual PVWML rating were 0.5, 2, and 5. The SCWML load had a first quintile, median and fourth quintile of 0.0, 0.3, and 2.4 ml.

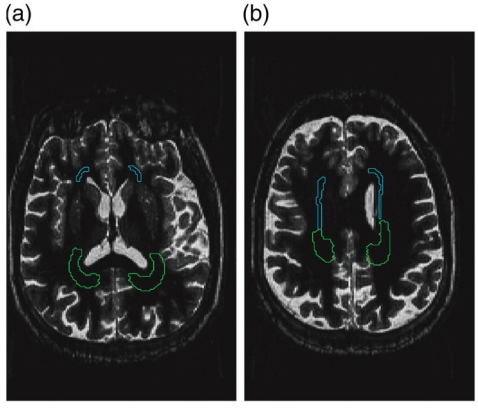

Figure 4. Two axial slices taken from one of the study images showing the anterior and posterior shells, in which the relation between blood pressure and regional WML volume was statistically significant at 0.05 after correction for multiple comparisons.

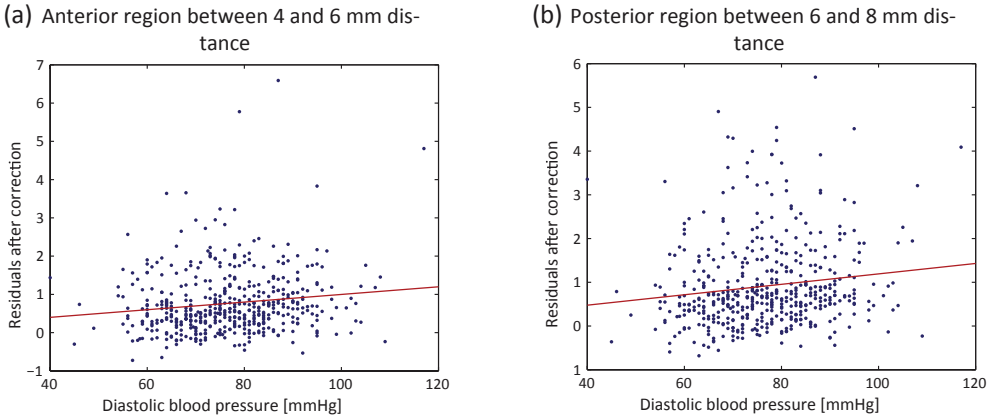

Figure 5. Scatter plots of the diastolic blood pressure versus the regional WML volume in two regions with a statistically significant association. The y-axis shows regional Z-scores after correction for age, sex, ICV, and use of blood-pressure-lowering medication. The red line represents the model computed with the regression analysis.

Table 2. Odds ratios of baseline blood pressure for severe PVWML and SCWML loads at follow-up (upper quintile versus the four lower quintiles combined), as measured with the visual rating and automated measurements.

	Visual	Automated
	PVWML	
Diastolic Blood Pressure	1.22 [0.99; 1.50]	1.12 [0.91; 1.38]
Systolic Blood Pressure	1.00 [0.88; 1.12]	1.06 [0.94; 1.20]
	SCWML	
Diastolic Blood Pressure	1.30 [1.05; 1.59]	1.25 [1.02; 1.54]
Systolic Blood Pressure	1.10 [0.98; 1.25]	1.06 [0.94; 1.19]

Values are odds ratios per 10 mmHg increase in blood pressure (95% CI). Adjusted for age, sex, intracranial volume, and the use of blood-pressure-lowering drugs.

The automated method measured a PVWML load with median and outer quintiles of 1.5, 5.2, and 13.1 ml, and median and outer quintile SCWML loads of 1.0, 3.8, and 10.4 ml. The difference between the SCWML volumes found by the automated method and the visual rating can be explained by the fact that the T2w and PDw images had a slice thickness of 5 mm. This effectively made the z-extent of all lesion voxels equal to approximately 5 mm. On the other hand, the visual scores assumed a spherical shape and were therefore generally smaller in size. Furthermore, in some cases cortical gray matter voxels were mislabeled as lesions by the automated method.

Table 2 shows the association between blood pressure and a severe load for PVWML and SCWML (upper quintile of the population distribution) as measured with the visual and automated ratings.

The two methods found similar relations between higher diastolic blood pressure and severe WML load in both regions, significant only for SCWML. Neither method showed an association between systolic blood pressure and the presence of a severe WML load.

DISCUSSION AND CONCLUSION

This section is divided in three parts: first the results of the regional WML analysis are interpreted and compared to the existing literature. In the second part, the method itself will be discussed and compared to alternative techniques. The section ends with the conclusions.

Discussion of the analysis

The main finding of the automated regional WML analysis is that the areas most affected by diastolic blood pressure are located between 4 and 6 mm in the anterior section, and between 4 and 10 mm in the posterior section. These regions are all located in the so-called vascular watershed area around the ventricles, supporting the notion that this area is especially vulnerable to ischemia.[4,27]

The WML load in the shell directly adjacent to the ventricles is not significantly related to diastolic blood pressure, although it contains the largest number of lesion voxels. From this result we conclude that WMLs in these areas can be found across the whole aging population, regardless of blood pressure. This hypothesis is in agreement with two pathological studies,[28,29] which suggested that small caps and pencil-thin lining around the ventricles are part of the normal aging process.

We also did not find evidence for an association between diastolic blood pressure and WML load further away from the ventricles. The association strength and level of significance dropped gradually when moving beyond the 4 mm boundary around the ventricles. This result could suggest that the presence of focal lesions outside the vascular watershed area is not heavily influenced by blood pressure, or that our method lacks power to detect this association.

The results can also be compared to the associations found with the commonly used distinction between periventricular and subcortical WMLs. We found a significant association between diastolic blood pressure and the risk of having a severe (upper quintile) SCWML load, and a borderline significant association with the risk of having a severe PVWML load. The associations were similar for both visual and automated PVWML/SCWML scores. The vascular watershed area that contains the confluent lesions falls in between the periventricular and subcortical zones. It seems likely that these same large lesions drive the association for both regions. The fact that blood pressure shows an association with both

PVWML and SCWML suggests that this hard distinction is too crude to study the influence of lesion location on the etiology.

Several groups have previously studied the relation between blood pressure and local WML load. For example a meta-analysis of ten European studies that used visual rating scales found associations between blood pressure and PVWML or SCWML load that were comparable to what is reported in this work.[30]

Yoshita and co-authors studied the relation between hypertension and the WML load in six small regions around the ventricles. In the three smallest regions the WML volume was not significantly higher for subjects with hypertension.[10] Two of these were directly adjacent to the ventricles. The three regions that did show a big increase also contained parts of the vascular watershed area. These results are therefore in accordance with most of our findings.

Enzinger and co-authors investigated the difference between lesion probability maps of subjects with and without hypertension.[11] Their analysis did not reveal voxel locations in the watershed areas that were significantly related to hypertension. This difference with our results may be caused by a lack of power needed to do a voxel-based analysis further away from the ventricles (this point is discussed in more detail below). However, the amount of persons studied in that paper was 189, less than half the size of the population considered in this work.

Evaluation of the method

The results demonstrate that the proposed method can observe distinctly different associations with the WMLs lining the ventricles, located in the vascular watershed area, and further out in the subcortical white matter. Moreover, we could observe different spatial distributions for WMLs in the central, anterior and posterior sections. This was impossible with WML measurements in the periventricular and subcortical regions only. In particular, confluent lesions in the watershed area cannot be accurately modeled by the simple threshold of the ventricular distance function that is used in automated PVWML/SCWML measurements. A hard distinction between these two types of lesions might therefore be too crude to shed further light on the debate about the role of WML location and might explain some of the ambiguous findings of previous work.

Furthermore, histopathological studies have found that small lesions lining the ventricles show a different pathology from severe periventricular lesions, and that confluent subcortical WMLs are distinct from focal lesions (See Gouw et al.[5] and Kim et al.[28] for an overview). The proposed method can better distinguish these different types and therefore allows further study of their etiology. Finally, unlike visual ratings our method is fully automated and does not suffer from inter- and intrarater variability. Compared to other automated methods it does not rely on an arbitrary border between the periventricular and subcortical white matter.

Our method has some limitations that need to be addressed. First of all, it does not measure WML load in regions defined in a template space. Most regional WML analyses[4,8,11,12] use registration to a common template to compensate for differences in head size which will affect the distance to the ventricles. Because we performed our analyses in each subject's native coordinate system, these differences might have caused additional variance on distance measurements and weakened the associations.

On the other hand, registration to a template introduces errors. The region segmentation applied in this work is also not immune to registration errors, but it allows the use of multiple atlases to compensate for this.[22] Furthermore, WMLs can negatively influence the registration process, as severe lesions tend to have higher signal intensities on the HASTE scan and appear as cerebrospinal fluid. Some papers have tried to address this problem by masking lesion areas[4] or basing the registration on the ventricles only.[12] But these methods will invariably introduce some uncertainty on the deformation of the lesions. Finally, it is unclear whether the exact location of the watershed area scales with head size, or whether it is determined by its absolute distance to the closest vessels.

As can be seen in Figure 5, the residuals of the multivariate linear model are not normally distributed. We therefore used permutation tests to compute p-values, which do not assume normality. Figure 5 suggests that the skewness predominantly affected the intercept and not the slope. Robust regression techniques like least absolute deviations could be used to compute a median slope, which could be addressed in a future work.

The experiments were based on MR scans acquired between 1995 and 1996. Although many more neuroimaging studies use data that is comparable to ours (see example http://adni.loni.ucla.edu/research/ protocols/mri-protocols/ for the MRI data used by the Alzheimer's Disease Neuroimaging Initiative), MRI technology has developed and therefore our images are not representative of the current state of the art. In particular, 3T scanners are increasingly being used and we did not have access to a FLAIR scan, which is well-suited to detect WMLs. Both developments have been shown to increase the sensitivity of detection of multiple sclerosis lesions.[31,32] However, we do believe that the results from our experiments can be generalized to more recent MR sequences, since our method is based on commonly used segmentation techniques like multi-atlas segmentation and voxel classification which have been applied to 3T data before.[33-35]

The increased sensitivity of 3T scanners and FLAIR sequences will most likely affect the trade-off between spatial resolution and the statistical power of our method. The improved detection of WML caused by better contrast and resolution allows the definition of smaller regions and consequently, better spatial localization of WML load. This could for example be achieved by decreasing the width of the shells, distinguishing the left and right hemisphere, or merging a smaller amount of atlas regions. Finding this new trade-off is comparable to choosing the size of the smoothing kernel in voxel-based morphometry.[36]

Finally, the spatial resolution of the proposed regional method is limited compared to a voxel-based analysis of WML probability maps. However, although lesion probability maps are a valuable tool to map the spatial WML distribution, we believe they are less suited to test associations involving regional WML load. Even for relatively large regions and in a population of 490 subjects, we obtained only a few associations that were significant after correcting for multiple comparisons. This might be the reason that previous studies that used lesion maps for this purpose found very few areas of significant association.[11,12] To the best of our knowledge, only one study based on probability maps has found associations not directly adjacent to the ventricles.[37]

Conclusion

This paper presented an automated method for measurement of the local white matter lesion load, based on the distance and orientation to the ventricles. The method allows a more spatially detailed analysis of the influence of lesion location. We demonstrated the potential of the method by studying the relation between blood pressure and regional WML load in 490 subjects, and found that diastolic blood pressure is related to an increased lesion load in the vascular watershed area on the border between the periventricular and subcortical zones. This result is compatible with the associations that we found for both severe PVWML and SCWML load computed for the same data. However, the proposed method can locate the area of association with a greater precision than methods that distinguish PVWMLs and SCWMLs only.

APPENDIX A

The anterior, posterior and central sections were composed from 83 structures and lobes defined according to Gousias et al.[20] This composition can be found in Table 3.

Table 3. Section composition based on the regions of interest defined in Goussias et al.[20]

Number in atlas	Name
Central	
20; 21	Insula
24; 25	Cingulate gyrus, anterior part
26; 27	Cingulate gyrus, posterior part
34; 35	Caudate nucleus
36; 37	Nucleus accumbens
38; 39	Putamen
40; 41	Thalamus
42; 43	Pallidum
44	Corpus callosum
45; 46	Lateral ventricle (excluding temporal horn)
49	Third ventricle
76; 77	Subgenual frontal cortex
78; 79	Subcallosal area
Anterior	
28; 29	Middle frontal gyrus
52; 53	Straight gyrus
54; 55	Anterior orbital gyrus
56; 57	Inferior frontal gyrus
58; 59	Superior frontal gyrus
68; 69	Medial orbital gyrus
70; 71	Lateral orbital gyrus
72; 73	Posterior orbital gyrus
80; 81	Pre-subgenual frontal cortex
Posterior	
22; 23	Lateral remainder of occipital lobe
30; 31	Posterior temporal lobe
32; 33	Inferiolateral remainder of parietal lobe
50; 51	Precentral gyrus
60; 61	Postcentral gyrus
62; 63	Superior parietal gyrus
64; 65	Lingual gyrus
66; 67	Cuneus
Excluded	
1; 2	Hippocampus
3; 4	Amygdala
5; 6	Anterior temporal lobe, medial part
7; 8	Anterior temporal lobe, lateral part
9; 10	Parahippocampal and ambient gyri
11; 12	Superior temporal gyrus, posterior part right
13; 14	Middle and inferior temporal gyrus
15; 16	Fusiform gyrus
17; 18	Cerebellum
19	Brainstem
47; 48	Lateral ventricle, temporal horn right
82; 83	Superior temporal gyrus, anterior part
74; 75	Substantial nigra left

REFERENCES

1. de Groot, J.C. *et al.* Cerebral white matter lesions and depressive symptoms in elderly adults. *Arch Gen Psychiatry* **57**, 1071-6 (2000).
2. de Groot, J.C. *et al.* Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. *Ann Neurol* **47**, 145-51 (2000).
3. Sachdev, P., Wen, W., Chen, X. & Brodaty, H. Progression of white matter hyperintensities in elderly individuals over 3 years. *Neurology* **68**, 214-22 (2007).
4. DeCarli, C., Fletcher, E., Ramey, V., Harvey, D. & Jagust, W.J. Anatomical mapping of white matter hyperintensities (WMH): exploring the relationships between periventricular WMH, deep WMH, and total WMH burden. *Stroke* **36**, 50-5 (2005).
5. Gouw, A.A. *et al.* Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. *J Neurol Neurosurg Psychiatry* **82**, 126-35 (2011).
6. Fazekas, F., Chawluk, J.B., Alavi, A., Hurtig, H.I. & Zimmerman, R.A. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol* **149**, 351-6 (1987).
7. Scheltens, P. *et al.* A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. *J Neurol Sci* **114**, 7-12 (1993).
8. Wen, W. & Sachdev, P. The topography of white matter hyperintensities on brain MRI in healthy 60- to 64-year-old individuals. *Neuroimage* **22**, 144-54 (2004).
9. van der Lijn, F., Vernooij, M.W., Ikram, M.A., Vrooman, H., Rueckert, D., Hammers, A., Breteler, M.M.B., Niessen, W.J. Automated localization of periventricular and subcortical white matter lesions. in *SPIE Medical Imaging 2007: Image Processing* (ed. Reinhardt, J.P.W.P., Joseph, M.) (2007).
10. Yoshita, M. *et al.* Extent and distribution of white matter hyperintensities in normal aging, MCI, and AD. *Neurology* **67**, 2192-8 (2006).
11. Enzinger, C. *et al.* Lesion probability maps of white matter hyperintensities in elderly individuals: results of the Austrian stroke prevention study. *J Neurol* **253**, 1064-70 (2006).
12. Jongen, C. *et al.* Construction of periventricular white matter hyperintensity maps by spatial normalization of the lateral ventricles. *Hum Brain Mapp* **30**, 2056-62 (2009).
13. Hofman, A. *et al.* The Rotterdam Study: 2010 objectives and design update. *Eur J Epidemiol* **24**, 553-72 (2009).
14. Ikram, M.A. *et al.* The Rotterdam Scan Study: design and update up to 2012. *Eur J Epidemiol* (2011).
15. den Heijer, T. *et al.* Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. *Diabetologia* **46**, 1604-10 (2003).
16. Sled, J.G., Zijdenbos, A.P. & Evans, A.C. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging* **17**, 87-97 (1998).
17. Ikram, M.A. *et al.* Brain tissue volumes in the general elderly population. The Rotterdam Scan Study. *Neurobiol Aging* **29**, 882-90 (2008).
18. Vrooman, H.A. *et al.* Multi-spectral brain tissue segmentation using automatically trained k-Nearest-Neighbor classification. *Neuroimage* **37**, 71-81 (2007).
19. Klein, S., Staring, M., Murphy, K., Viergever, M.A. & Pluim, J.P. elastix: a toolbox for intensity-based medical image registration. *IEEE Trans Med Imaging* **29**, 196-205 (2010).
20. Gousias, I.S. *et al.* Automatic segmentation of brain MRIs of 2-year-olds into 83 regions of interest. *Neuroimage* **40**, 672-84 (2008).
21. van der Lijn, F., den Heijer, T., Breteler, M.M. & Niessen, W.J. Hippocampus segmentation in MR images using atlas registration, voxel classification, and graph cuts. *Neuroimage* **43**, 708-20 (2008).
22. Heckemann, R.A., Hajnal, J.V., Aljabar, P., Rueckert, D. & Hammers, A. Automatic anatomical brain MRI segmentation combining label propagation and decision fusion. *Neuroimage* **33**, 115-26 (2006).
23. Freedman, D., Lane, D. A nonstochastic interpretation of reported significance levels. *J. Bus. Econ. Stat.* **1**, 292–298 (1983).
24. Anderson, M.J., Robinson, J. Permutation tests for linear models. *Aust. N. Z. J. Stat.* **43**, 75–88 (2001).
25. Nichols, T.E. & Holmes, A.P. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum Brain Mapp* **15**, 1-25 (2002).

26. de Leeuw, F.E. *et al.* A follow-up study of blood pressure and cerebral white matter lesions. *Ann Neurol* **46**, 827-33 (1999).
27. Moody, D.M., Bell, M.A. & Challa, V.R. Features of the cerebral vascular pattern that predict vulnerability to perfusion or oxygenation deficiency: an anatomic study. *AJNR Am J Neuroradiol* **11**, 431-9 (1990).
28. Fazekas, F. *et al.* Pathologic correlates of incidental MRI white matter signal hyperintensities. *Neurology* **43**, 1683-9 (1993).
29. Takao, M. *et al.* Pathologic findings of silent hyperintense white matter lesions on MRI. *J Neurol Sci* **167**, 127-31 (1999).
30. van Dijk, E.J. *et al.* The association between blood pressure, hypertension, and cerebral white matter lesions: cardiovascular determinants of dementia study. *Hypertension* **44**, 625-30 (2004).
31. Simon, J.H. *et al.* Standardized MR imaging protocol for multiple sclerosis: Consortium of MS Centers consensus guidelines. *AJNR Am J Neuroradiol* **27**, 455-61 (2006).
32. Wattjes, M.P. *et al.* Double inversion recovery brain imaging at 3T: diagnostic value in the detection of multiple sclerosis lesions. *AJNR Am J Neuroradiol* **28**, 54-9 (2007).
33. Gibson, E., Gao, F., Black, S.E. & Lobaugh, N.J. Automatic segmentation of white matter hyperintensities in the elderly using FLAIR images at 3T. *J Magn Reson Imaging* **31**, 1311-22 (2010).
34. Heckemann, R.A. *et al.* Automatic morphometry in Alzheimer's disease and mild cognitive impairment. *Neuroimage* **56**, 2024-37 (2011).
35. Lotjonen, J. *et al.* Fast and robust extraction of hippocampus from MR images for diagnostics of Alzheimer's disease. *Neuroimage* **56**, 185-96 (2011).
36. Ashburner, J. & Friston, K.J. Diffeomorphic registration using geodesic shooting and Gauss-Newton optimisation. *Neuroimage* **55**, 954-67 (2011).
37. Taylor, W.D. *et al.* Localization of age-associated white matter hyperintensities in late-life depression. *Prog Neuropsychopharmacol Biol Psychiatry* **27**, 539-44 (2003).

Chapter 2.2

Changes in normal-appearing white matter precede development of white matter lesions

Marius de Groot*, Benjamin F.J. Verhaaren*, Renske de Boer, Stefan Klein,
Albert Hofman, Aad van der Lugt, M. Arfan Ikram, Wiro J. Niessen, Meike W. Vernooij.
*contributed equally

Stroke. 2013 Apr;44(4):1037-42.

ABSTRACT

Background and Purpose: It is unknown whether white matter lesions (WMLs) develop abruptly in previously normal brain areas, or whether tissue changes are already present before WMLs become apparent on MRI. We therefore investigated whether development of WMLs is preceded by quantifiable changes in normal-appearing white matter (NAWM).

Methods: In 689 participants from the general population (mean age 67 years) we performed two MRI scans (including DTI and FLAIR sequences) 3.5 years apart using the same 1.5-T scanner. Using automated tissue segmentation, we identified NAWM at baseline. We assessed which NAWM-regions converted into WMLs during follow-up, and differentiated new WMLs into regions of WML growth and de-novo WMLs. Fractional anisotropy (FA), mean diffusivity (MD) and FLAIR-intensity of regions converting to WMLs and regions of persistent NAWM were compared using three approaches: a whole-brain analysis, a regionally matched approach and a voxel-wise approach.

Results: All three approaches showed that low FA, high MD and relatively high FLAIR-intensity at baseline were associated with WML development during follow-up. Compared to persistent NAWM-regions, NAWM-regions converting to WMLs had significantly lower FA (0.337 vs. 0.387, $p<0.001$), higher MD ($0.910*10^{-3}$ mm^2/s vs. $0.729*10^{-3}$ mm^2/s, $p<0.001$) and relatively higher normalized FLAIR-intensity (1.233 vs. -0.340, $p<0.001$). This applied to both NAWM developing into growing and de-novo WMLs.

Conclusions: White matter changes in NAWM are present and can be quantified on DTI and FLAIR before WMLs develop. This suggests that WMLs develop gradually and that visually appreciable WMLs are only the tip of the iceberg of white matter pathology.

INTRODUCTION

Cerebral white matter lesions (WMLs) in the elderly are frequently seen on MRI. They are considered to reflect subclinical vascular brain disease and are associated with an increased risk of dementia and stroke.[1,2] Preventing or slowing down WML development may thus have the potential to decrease disease burden. To date, several potentially modifiable risk factors, such as smoking and high blood pressure, have been associated with WML development.[1,3] Yet, the pathogenesis of WMLs is still poorly understood. Most importantly, it is unknown whether WMLs develop abruptly in previously normal brain regions or whether development of WMLs on MRI is a gradual process, in which tissue changes are already present before they become apparent on MRI as WMLs. This is especially important in order to identify in which persons and at what moment preventive measures should be installed.

On MRI, WMLs are best visualized by the Fluid Attenuation Inversion Recovery (FLAIR) sequence on which WMLs appear as hyper-intense regions in the white matter. WMLs can be quantified using visual rating scales or automated measurements. Both methods measure *visually appreciable* WMLs, i.e. the macrostructural changes of the white matter that are clearly distinguished on a FLAIR scan. However, pathology studies suggest that these WMLs are only the tip of the iceberg of white matter pathology.[4] If WML development is indeed a gradual process, early stages of its development might be accompanied by subtly increased FLAIR intensities.

Diffusion tensor imaging (DTI) is a relatively recent MR imaging technique that allows in-vivo study of tissue microstructure and is often applied to study cerebral white matter. DTI provides multiple imaging metrics such as fractional anisotropy (FA) and mean diffusivity (MD). These metrics have been shown to detect changes in white matter microstructure that are not distinguished on conventional MRI.[5] Performing DTI in longitudinal MR imaging studies enables investigation of normal-appearing white matter (NAWM) microstructure before WMLs develop.[6]

New WMLs form either as lesion growth (i.e. adhering to already present WMLs) or as "de-novo" WMLs. It is important to take this distinction into account. First, this allows investigation of potentially different etiologies. Second, around the border of existing WMLs, NAWM voxels can contain a fraction of WML tissue that affects measurements in that voxel (so-called partial volume effect). This introduces a potential bias, which is not present for de-novo WMLs.

It is also important to take into account that DTI measurements vary considerably across brain regions due to neuronal tract-width and tract-geometry, and that WMLs preferentially occur in specific brain regions. These observations demand that longitudinal analyses of NAWM features and WML development take spatial location into account. Finally, to investigate the generic pathophysiology of WML development this should preferably be studied in the general population.

Therefore, in 689 participants from the population-based Rotterdam Scan Study, we investigated whether DTI measures and the FLAIR intensity of NAWM at baseline are associated with growing WMLs and de-novo WMLs over a period of 3.5 years. To take into account the lesion location of measurements in the white matter, we used regional matching and a voxel-based approach.

METHODS

Study population

This study is based on participants from a large, prospective, population-based cohort study in the Netherlands that investigates determinants of various chronic diseases in elderly people.[7] The original study population consisted of 7,983 people in the general population; aged 55 years and older and all residents of the Ommoord suburb of Rotterdam. In 2000 to 2001, the cohort was expanded with 3,011 people aged 55 years and over.[7] The institutional review board approved the study, and written informed consent was obtained from all participants.

In 2005, 1,073 from these 3,011 people were randomly selected for MRI-scanning.[8] After exclusion of demented people (N=4) and people that had MRI contraindications (N=94), 975 were eligible, of whom 907 (93%) participated. Physical inabilities precluded image acquisition in 12 individuals. Imaging was incomplete for 3 subjects, leaving 892 people with complete MRI examinations. In 2008, these people where invited for a follow-up MRI-scan. After exclusion of people that had died (N=21) or had new MRI contraindications (N=7), 864 people were eligible. From these, 770 (89%) were willing to participate of whom 754 had complete MRI-examinations. After exclusion of people with cortical infarcts at either baseline or follow-up (N= 32), 722 people were included in this study.

MRI protocol

The MRI protocol performed at both time-points was identical and was performed on the same 1.5T GE Signa Excite MR scanner in a standardized way. Details of this protocol have been described elsewhere.[8] In short, structural imaging included a T1-weighted 3D Fast RF Spoiled Gradient Recalled Acquisition in Steady State with an inversion recovery pre-pulse (FASTSPGR-IR) sequence, a proton density (PD) weighted sequence, and a T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence. For DTI, we performed a single shot, diffusion-weighted spin echo echo-planar imaging sequence. Maximum b-value was 1000 s/mm2 in 25 non-collinear directions; one volume was acquired without diffusion weighting (b-value = 0 s/mm2).

Tissue segmentation

Brain tissue was classified into NAWM, WML, grey matter and cerebrospinal fluid. For classification of all tissues except WMLs, a multi-spectral tissue classification[9] was used, incorporating a multi-atlas strategy with 6 manually labeled atlases for learning subject-specific tissue intensities. The FLAIR intensity was used to identify WMLs in an automated post-processing step.[10] Tissue segmentations were visually inspected. Subjects with artifacts in the segmentation of either scan (e.g. due to motion) were excluded (33), leaving 689 subjects for analysis.

Spatial and intensity normalization

Non-rigid image registration, using FLIRT[11] and FNIRT,[12] was used to align the T1w structural images of both time-points. In order to prevent biasing towards a particular time-point,[13] both scans were transformed to the (subject-specific) intermediate space by inverting half the

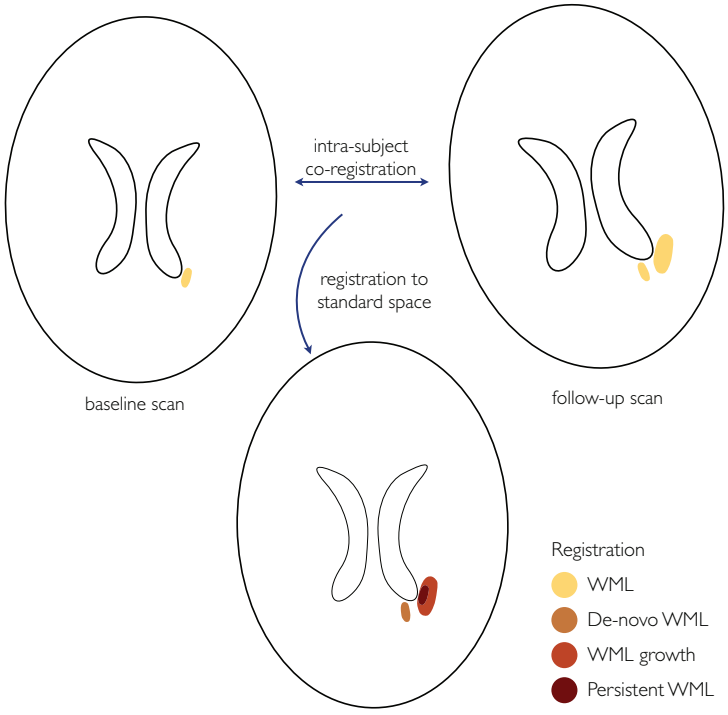

Figure 1. Schematic overview of the spatial normalization procedure for two scans of the same subject.

The baseline and follow-up scans are nonlinearly registered towards one another allowing creation of an intermediate image. This intermediate image is then non-linearly registered to standard space. WML segmentations for both scans, transformed to standard space, are automatically categorized into persistent WML and new WML. New WML are further distinguished into WML growth if the new lesion is connected to a baseline WML or de-novo WML if it is not. Abbreviation: WML = white matter lesion.

deformation fields of the transformations between both scans. For each subject, the mean intermediate T1w images were registered to the 1mm MNI_152 template, supplied with the FSL toolbox[14] (version 4.1) using FNIRT. A schematic overview of the spatial normalization process is given in Figure 1. WMLs were masked to minimize their influence on the registration. FLAIR intensities were normalized across subjects by matching grey matter intensity histograms for each subject (matching peak and full width at half maximum using linear transformations). This normalization was driven by grey matter intensities to avoid potential influence of subclinical white matter pathology. Non-uniformity correction (prior to normalization) and co-registration to the T1w image were performed as described in.[10]

Diffusion data processing

Diffusion data were corrected for motion and eddy currents by affine co-registration of the diffusion weighted volumes to the b=0 volume. Registrations were performed with Elastix.[15] The rotation component of each transformation was used to realign each gradient vector to compensate for motion during the acquisition.[16] Transformed diffusion weighted images were resampled at an isotropic resolution of 1.0 mm. The Brain Extraction Tool[17] from FSL was used to mask out non-brain tissue. Tensor fits were performed with a Levenberg-Marquard non-linear least squares optimization algorithm, available in ExploreDTI.[18] Data quality was examined by visual inspection of axial FA slices, every 4 mm, combined with two coronal and two sagittal slices around the center of the brain. Resampling of diffusion data in standard space was performed in one pass by concatenating an affine co-registration of the FA to the baseline T1w image, the nonlinear transformations of T1w space to mean structural space, and the transformation from that space to standard space. All registrations were checked by visually inspecting the warped structural and FA images in standard space. No unacceptable misregistrations were found.

Definition of new WML, growing and de-novo WML

WMLs were defined as each group of voxels, classified as WML in the tissue segmentation, connected in a 3-dimensional 18-voxel-neighborhood (spherical kernel with a diameter of 3 voxels). In standard space, brain tissue segmentations for both time points were combined per subject to obtain voxelwise 'persistent NAWM', 'persistent WML' and 'new WML' (i.e. converting from NAWM to WML) tissue classes (Figure 1). Every WML in the follow-up image was then checked for overlap with WMLs in the baseline image. The new WML voxels were subdivided into 'WML growth' and 'de-novo' WML tissue according to this overlap. New WML voxels were identified as WML growth if they were part of a WML that overlapped with a WML at baseline. Accordingly, if not overlapping with a baseline WML, new WML voxels were classified as de-novo WML.

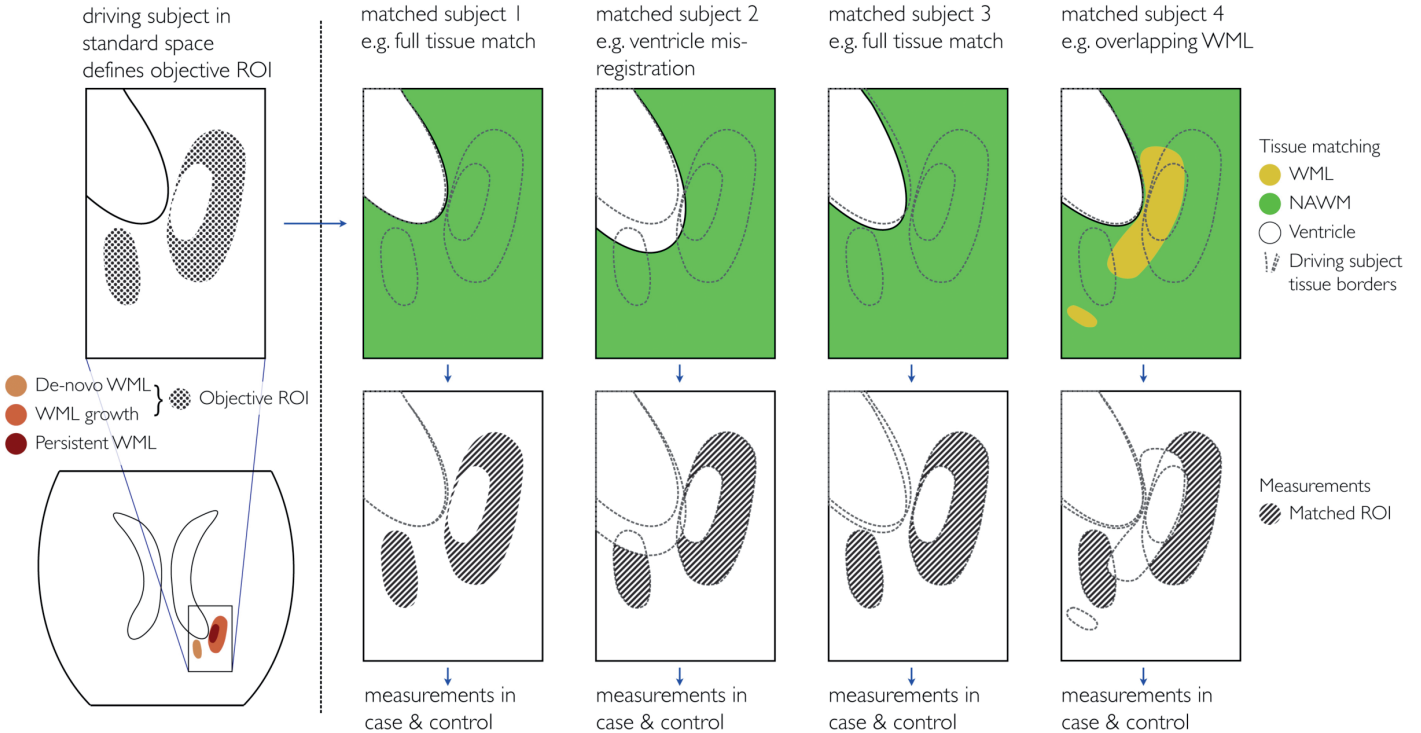

Figure 2. Schematic overview of the regional matching procedure, illustrated with enlarged axial cutouts for 5 subjects.

The left panel shows how the new WML in standard space for the driving subject are treated as objective region of interest (ROI). This objective ROI is distinguished into WML growth and de-novo WML (not shown). For age and gender matched subjects in the same population, the overlap between the matched subject NAWM and the objective ROI is determined. The matching is performed four times to increase robustness, e.g. to be robust against a situation where new lesions in the driving subject overlap with WML in the matched subject. The repetition also means that every subject is driving-subject once, and matched subject exactly four times in the analysis. In the graph, matched subjects 1 and 3 show a complete overlap between the objective ROI and the persistent NAWM. For subject 2, the enlarged ventricle is not perfectly registered, leading to part of the ventricle overlapping with the objective ROI. Subject 4 shows WML overlapping with the objective ROI. The unmatched regions are excluded from the objective ROI in calculating the average baseline DTI and FLAIR metrics. The four resulting measurement pairs for each metric are averaged across the pairs. Abbreviations: WML = white matter lesion, NAWM = normal-appearing white matter, ROI = region of interest, DTI = diffusion tensor imaging, FLAIR = fluid attenuated inversion recovery.

Regional matching

A schematic overview of the approach is given in Figure 2. To take into account that diffusion metrics and WML formation depend on anatomical location, we performed an analysis in which regions developing into WML were compared to anatomically corresponding regions of persistent NAWM. Regional matching was completed by confining measurements to the overlap between new WML in the driving subject and persistent NAWM in the matched subject to account for registration errors and potential (new) WMLs in the matched subject in those locations. In those regions in standard space, we averaged FLAIR and DTI metrics in the baseline scans. This process was repeated four times with different age and sex matched subjects for each driving subject for additional robustness.

Statistical analyses

We investigated baseline tissue properties of NAWM developing into WML during follow-up using three approaches. First, we performed a whole-brain analysis without regional matching. We averaged FLAIR intensity and diffusion metrics in all persistent NAWM voxels, and compared these to measures inside NAWM converting into WML, further partitioned into WML growth and de-novo WML. Hereto, we used paired-samples t-tests (two sided, α-value = 0.05) using SPSS statistical software (version 20).

Second, we used the regional matching to compare baseline measurements in NAWM developing into WML with those in regionally matched persistent NAWM of age- and sex-matched controls. Persistent NAWM and WML measurements were averaged across the four repetitions in order to generate the measurement pairs to be used in paired-samples t-tests. To test the added information of DTI metrics over FLAIR intensity and vice versa, we added measures from both modalities as independent variables, and fitted a conditional logistic regression model with new lesion status as outcome measure and DTI or FLAIR measurements in baseline NAWM as determinant.

Third, to investigate regional dependence of the associations, we tested for voxel-wise differences in diffusion and FLAIR measurements between new WML and persistent NAWM. For each voxel, a regression was performed using the metric of interest as dependent variable, and age and lesion status as (voxelwise) independent variables provided that the each tissue class was represented by at least 10 subjects. This constraint effectively limited the analysis to the periventricular watershed area. Analyses were performed using t-tests in Randomise,[19] available in FSL, using 5000 permutations to correct for multiple comparisons (α-value = 0.05). Threshold free cluster enhancement (TFCE)[20] was used to cluster significant results. We repeated the test for added information of diffusion metrics over FLAIR intensity and vice versa on a voxel-wise level by adding measures from both modalities as voxel-wise independent variable in the same model.

RESULTS

Characteristics of the study population are presented in Table 1. The median WML volume at baseline was 3.4 mL. After on average 3.5 years of follow up, we observed a net increase in WML volume in 81% of the participants, and a net decrease in WML volume in the remaining 19% of the participants (median increase 1.4 mL, loss 0.8 mL). Table 2 represents the DTI and FLAIR parameters of persisting NAWM versus NAWM converting to WMLs for the whole brain analysis. Compared to persistent NAWM regions, NAWM regions converting to WML had significantly lower FA (0.337 (standard deviation: 0.030) vs. 0.387 (standard deviation: 0.017), p<0.001), higher MD (0.910 * 10^{-3} (0.054*10^{-3}) mm^2/s vs. 0.729 *10^{-3} (0.027*10^{-3}) mm^2/s, p<0.001) and relatively higher normalized FLAIR intensity (1.233 (0.150) vs. -0.340 (0.190), p<0.001). This applied to both NAWM regions of growing and de-novo WMLs.

Table 1. Population characteristics

	N=689
Age, years	66.9 (5.0)
Female	52 % (355)
Baseline WML volume, mL*	3.4 [2.1 6.5]
New WML volume, mL*	1.4 [0.8 2.8]
de-novo WML volume, mL*	0.2 [0.1 0.3]
Growing WML volume, mL*	1.1 [0.6 2.4]
Lost WML volume, mL*	0.8 [0.5 1.3]
Baseline NAWM volume, mL	397 (53)
NAWM FA	0.387 (0.017)
NAWM MD, 10^{-3} mm^2/s	0.730 (0.028)
NAWM FLAIR	-.332 (.194)
Follow up time, years	3.5 (0.2)

Values are means (standard deviation) or percentages (numbers). *median [interquartile range]. Abbreviations: WML = white matter lesion, NAWM = normal-appearing white matter, FLAIR = Fluid Attenuated Inversion-Recovery (normalized signal intensity).

Table 2. DTI and FLAIR parameters of persisting NAWM versus NAWM converting to WMLs; whole brain analysis

	Persisting NAWM				NAWM converting to WMLs				
FA	0.387 (0.017)	(Ref)	0.337 (0.030)	p<0.001	0.335 (0.033)	p<0.001	0.346 (0.038)	p<0.001
MD, 10^{-3} mm^2/s	0.729 (0.027)	(Ref)	0.910 (0.054)	p<0.001	0.919 (0.055)	p<0.001	0.866 (0.073)	p<0.001
FLAIR	-0.340 (0.190)	(Ref)	1.233 (0.150)	p<0.001	1.295 (0.151)	p<0.001	0.920 (0.191)	p<0.001

Values are means (SD). p-values are based on the paired-samples t-test results of the comparisons of mean FA, MD and FLAIR values of persisting NAWM versus the values of NAWM converting to WMLs. Abbreviations: WMLs = white matter lesions, NAWM= normal-appearing white matter, FA = fraction anisotropy, MD = mean diffusivity, FLAIR = Fluid Attenuated Inversion Recovery (normalized signal intensity).

In Table 3 the difference in DTI and FLAIR parameters is shown between converting NAWM versus persisting NAWM with respect to the regionally matched analyses, using 698 sets of matched subjects. Again, compared to persistent NAWM regions, NAWM regions converting to WML had lower FA (difference (95%-confidence interval: -0.0327 (-0.0339; -0.0315), p<0.001), higher MD (0.0646 (0.0625; 0.0668) *10^{-3}mm^2/s, p<0.001) and relatively higher normalized FLAIR intensity (0.895 (0.884; 0.906), p<0.001). This also applied to both NAWM regions of growing and de-novo WMLs. In addition we found that low FA and the high normalized FLAIR-intensity were associated with WML development (growing and de-novo WMLs) after adjustment for one other, see Table 4. MD was not significantly associated with WML development after adjustment for the normalized FLAIR intensity.

Table 3. Difference in DTI and FLAIR parameters between persisting NAWM and NAWM converting to WMLs; regionally matched analysis

	Persisting NAWM	NAWM converting to WMLs					
		total		growing		de-novo	
FA	(Ref)	-0.0327 (-0.0339; -0.0315)	p<0.001	-0.0340 (-0.0353; -0.0326)	p<0.001	-0.0233 (-0.0246; -0.0219)	p<0.001
MD, 10^{-3} mm^2/s	(Ref)	0.0646 (0.0625; 0.0668)	p<0.001	0.0678 (0.0656; 0.0700)	p<0.001	0.0435 (0.0413; 0.0456)	p<0.001
FLAIR	(Ref)	0.895 (0.884; 0.906)	p<0.001	0.942 (0.931; 0.953)	p<0.001	0.682 (0.671; 0.693)	p<0.001

Values are mean differences (95%-CI) of FA, MD or normalized FLAIR signal intensity of NAWM converting regions and NAWM persisting regions, matched on age, sex and anatomical region. p-values are based on paired-samples t-tests. NAWM = normal-appearing white matter, FA = fraction anisotropy, MD = mean diffusivity, FLAIR = Fluid Attenuated Inversion Recovery (normalized signal intensity).

Table 4. Odds Ratios of WML development per SD increase in baseline DTI and FLAIR parameters of NAWM

	total		growth		de-novo	
MODEL I						
FA	0.31 (0.29; 0.34)	p<0.001	0.33 (0.30; 0.36)	p<0.001	0.42 (0.39; 0.46)	p<0.001
MD	3.99 (3.66; 4.35)	p<0.001	3.85 (3.54; 4.19)	p<0.001	2.83 (2.61; 3.08)	p<0.001
FLAIR	25.96 (19.37; 34.79)	p<0.001	25.45 (19.05; 34.01)	p<0.001	17.65 (14.36; 21.69)	p<0.001
MODEL II						
FA	0.84 (0.74; 0.95)	p=0.008	0.78 (0.69; 0.88)	p<0.001	0.81 (0.72; 0.92)	p<0.001
MD	1.19 (1.03; 1.39)	p=0.02	1.42 (1.22; 1.65)	p<0.001	1.04 (0.95; 1.15)	p=0.4
FLAIR*	23.99 (17.66; 32.58)	p<0.001	23.97 (17.48; 32.87)	p<0.001	16.75 (13.55; 20.69)	p<0.001

Values are Odds Ratios (95%-CI) per SD increase in FA, MD or normalized FLAIR signal intensity.
Model I: conditional logistic regression with age, sex and region matched converting and persisting NAWM regions, adjusted for time between scans.
Model II: as Model I with additional adjustment for normalized FLAIR signal intensity. *adjusted for FA and MD.
Abbreviations: NAWM = normal-appearing white matter, FA = fraction anisotropy, MD = mean diffusivity, FLAIR = Fluid Attenuated Inversion Recovery (normalized signal intensity).

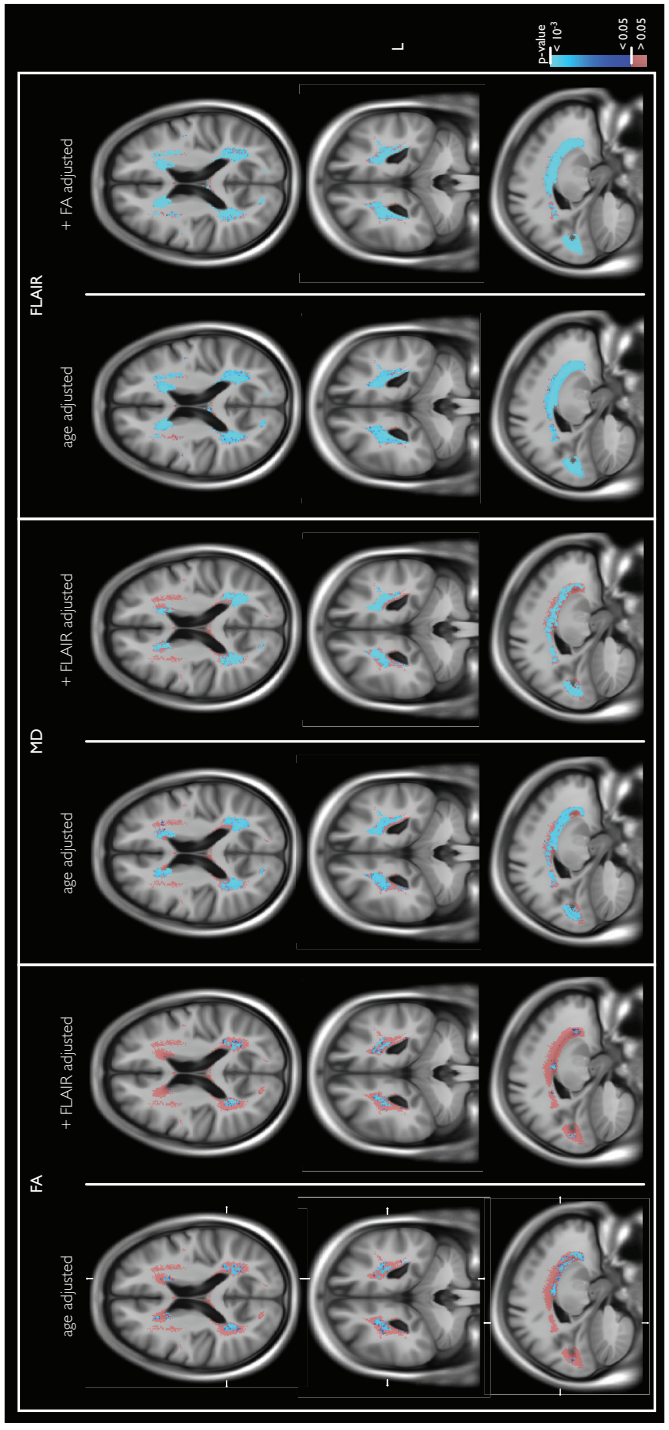

Figure 3. Voxel-wise comparison of baseline DTI measures and FLAIR intensity in NAWM converting to WML versus persisting NAWM.

Only voxels in which there were at least 10 subjects in both categories were analyzed (shown in red and blue). This effectively constrained the analysis to the periventricular watershed area. Shown in blue are voxels in which DTI and/or FLAIR measures at baseline were significantly related to WML development during follow up. For the FA, the left panel shows regions of significantly lower FA, corrected for age. The right panel shows the same analysis, additionally corrected for voxel-wise FLAIR intensity. Left panels in MD and FLAIR show regions of significantly higher MD or relatively higher normalized FLAIR intensity related to WML development adjusted for age. The right panel for the MD is additionally adjusted for FLAIR intensity. The right panel for the FLAIR is additionally adjusted for FA values. FLAIR adjusted for MD showed similar results (not shown). Abbreviations: WML = white matter lesion, NAWM = normal-appearing white matter, FA = fractional anisotropy, MD = mean diffusivity, DTI = diffusion tensor imaging, FLAIR = fluid attenuated inversion recovery.

Results for the voxel-wise analysis are displayed in Figure 3. All measures were indicative of deteriorated microstructure in the NAWM converting to WML compared to persistent NAWM. Differences were significant, bilaterally along the full span of the ventricles. However, using TFCE-analysis, cluster-size of the associations did vary for the different measures. The cluster for higher MD was broader than that for lower FA. Relatively higher normalized FLAIR intensity was significant in almost the entire analyzed region. Adjusting for alternate measures only slightly reduced significance, mostly visible in the FA analysis corrected for FLAIR intensity (Figure 3).

DISCUSSION

In this longitudinal MRI study over 3.5 years, we found that visually not appreciable but quantifiable changes of the white matter precede the development of WMLs. More specifically, we found that baseline DTI-measures and FLAIR signal intensity were associated with both growing WMLs (i.e., new WMLs adhering to already present WMLs at baseline) and de-novo WMLs (i.e., new WMLs not adhering to an already present WML at baseline). Furthermore, we found that DTI measures and FLAIR signal intensity were associated with WML development independently from each other.

Strengths of this study are its longitudinal design, large sample size, population-based setting and the use of the same scanner and imaging protocol at baseline and follow up. Additionally, we accounted for the spatial dependency of both WMLs and diffusion metrics in two ways, by using regional matching and a voxel-based approach. Furthermore, we distinguished growing WMLs and de-novo WMLs. This not only enabled us to study potential differences in etiology, but also contributed to the validity of our study, since analyses regarding de-novo WMLs are less likely to suffer from biases. For example, the dichotomization of segmenting voxels into WML and NAWM based on FLAIR intensity leads to a so-called partial-voluming effect in the voxels on the interface between both tissues. For de-novo WMLs, the absence of such an interface in the baseline NAWM avoids a potential partial-voluming bias for these lesions.

A limitation of our study is that, though we know which voxels have developed into WMLs over 3.5 years follow-up, we do not know at exactly which moment during follow-up these lesions developed; this may have been days, months or years after the baseline scan. Nevertheless, this does not change our primary observation that NAWM changes precede the appearance of visually appreciable WMLs. Another consideration is that the WML burden in our study was relatively low because of the population-based setting. Yet, we expect that our conclusions also extend to a patient population with high WML burden, since large WMLs have been reported to be surrounded by a penumbra of abnormal NAWM.[28]

We found an apparent net decrease of WML volume in 19% of our population, likely attributable to misclassification of tissues and measurement error at baseline or follow-up, which is in line with previous research.[21,22] As we performed our analyses in standard space, the increase or decrease of WML could also be assessed at a voxel level. This not only showed new WML voxels in all subjects, but also a loss of one or more WML voxels in all subjects, which again is likely to result from misclassification or measurement error in either time point. As these voxels did not qualify as new WML in our definition, they were not included in the analysis. Therefore, if anything, this misclassification will either not have influenced our results or may have led to a slight underestimation.

Only one other study reported on the relation between changes of the NAWM at baseline and the development of WMLs in a longitudinal MR study, but they did not distinguish between growing and de-novo WMLs.[6] In line with our findings, they found FA and FLAIR intensity to be independently associated with the development of WMLs in a heterogeneous population of 119 people with Alzheimer's disease, mild-cognitive impairment and normal cognitive function. Together with our findings, this further corroborates that WMLs are the result of a gradual process, as we now assess that this also applies to the general population, and that it holds for both growing as well as de-novo WMLs. In addition, using the voxelwise analysis, we found no evidence that this process is spatially varying along the ventricles.

WMLs in the elderly are considered to be mainly vascular in origin. This is based on numerous epidemiological studies that found vascular risk factors such as high blood pressure to be associated with WMLs, and pathology studies that found damage of the cerebral small vessels, signs of blood brain barrier dysfunction and ischemic pathology in WMLs.[1,23,24] Previous longitudinal studies have shown that baseline WML load is strongly associated with WML progression,[25,26] and cross-sectional studies found abnormalities in the NAWM to be related with WML burden,[4,27,28] Yet, it was unknown whether the disease-process develops gradually or abruptly. This information is essential, because if WMLs would develop abruptly, other causes would be more likely (e.g., acute ischemia) than when WMLs develop over a longer period (e.g., chronic ischemia). In addition it was unknown whether WML growth and de-novo WML development have a similar pathophysiology. Our findings suggest that both growing and de-novo WMLs develop gradually. Therefore a shared pathophysiological process is likely. Our results also suggest that DTI measures and FLAIR signal intensity provide some information independently from each other, implying that both measures partially capture different elements of tissue pathology. Furthermore, our findings confirm that WMLs are the extremes, or 'the tip of the iceberg', of white matter pathology.[4,25,27,28]

Our findings may have clinical implications. A clinician should take into account that the true white matter pathology may be more extensive than what is visually appreciable on structural MRI. This could lead to an improved estimate of a patients' risk of stroke,

dementia and death.[2,29] However, further research is needed to confirm these hypotheses. In summary, in this longitudinal MRI study we found normal-appearing white matter changes to be present before WMLs develop. This suggests that the pathophysiology of WMLs is a gradual process. Furthermore, our results suggest that WMLs are only the tip of the iceberg of white matter pathology.

REFERENCES

1. Pantoni L. Pathophysiology of age-related cerebral white matter changes. *Cerebrovascular diseases*. 2002;13 Suppl 2:7–10.
2. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ*. 2010;341:c3666.
3. Van Dijk EJ, Prins ND, Vrooman HA, Hofman A, Koudstaal PJ, Breteler MMB. Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences: Rotterdam Scan study. *Stroke*. 2008;39:2712–2719.
4. Simpson JE, Ince PG, Higham CE, Gelsthorpe CH, Fernando MS, Matthews F, et al. Microglial activation in white matter lesions and nonlesional white matter of ageing brains. *Neuropathology and applied neurobiology*. 2007;33:670–683.
5. Vernooij MW, De Groot M, Van der Lugt A, Ikram MA, Krestin GP, Hofman A, et al. White matter atrophy and lesion formation explain the loss of structural integrity of white matter in aging. *NeuroImage*. 2008;43:470–477.
6. Maillard P, Carmichael O, Harvey D, Fletcher E, Reed B, Mungas D, et al. FLAIR and Diffusion MRI Signals Are Independent Predictors of White Matter Hyperintensities. [Published online ahead of print June 14, 2012]. *AJNR*. 2012. doi:10.3174/ajnr.A3146
7. Hofman A, Van Duijn CM, Franco OH, Ikram MA, Janssen HLA, Klaver CCW, et al. The Rotterdam Study: 2012 objectives and design update. *European Journal of Epidemiology*. 2011;26:657–686.
8. Ikram MA, Van der Lugt A, Niessen WJ, Krestin GP, Koudstaal PJ, Hofman A, et al. The Rotterdam Scan Study: design and update up to 2012. *European Journal of Epidemiology*. 2011;26:811–824.
9. Vrooman HA, Cocosco CA, Van der Lijn F, Stokking R, Ikram MA, Vernooij MW, et al. Multi-spectral brain tissue segmentation using automatically trained k-Nearest-Neighbor classification. *NeuroImage*. 2007;37:71–81.
10. De Boer R, Vrooman HA, Van der Lijn F, Vernooij MW, Ikram MA, Van der Lugt A, et al. White matter lesion extension to automatic brain tissue segmentation on MRI. *NeuroImage*. 2009;45:1151–1161.
11. Jenkinson M, Smith SM. A global optimisation method for robust affine registration of brain images. *Medical Image Analysis*. 2001;5:143–156.
12. Andersson JLR, Jenkinson M, Smith SM. Non-linear registration aka Spatial normalisation. *FMRIB Technical Report TR07JA2*. 2007;
13. Reuter M, Fischl B. Avoiding asymmetry-induced bias in longitudinal image processing. *NeuroImage*. 2011;57:19–21.
14. Woolrich MW, Jbabdi S, Patenaude B, Chappell M, Makni S, Behrens T, et al. Bayesian analysis of neuroimaging data in FSL. *NeuroImage*. 2009;45:S173–186.
15. Klein S, Staring M, Murphy K, Viergever MA, Pluim JPW. elastix: a toolbox for intensity-based medical image registration. *IEEE Transactions on Medical Imaging*. 2010;29:196–205.
16. Leemans A, Jones DK. The B-matrix must be rotated when correcting for subject motion in DTI data. *Magn Reson Med*. 2009;61:1336–1349.
17. Smith SM. Fast robust automated brain extraction. *Human brain mapping*. 2002;17:143–155.
18. Leemans A, Jeurissen B, Sijbers J, Jones D. ExploreDTI: a graphical toolbox for processing, analyzing, and visualizing diffusion MR data. In: Proceedings 17th Scientific Meeting International Society for Magnetic Resonance in Medicine. 2009;3537.
19. Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum Brain Mapp*. 2002;15:1–25.
20. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *NeuroImage*. 2009;44:83–98.
21. Van den Heuvel DMJ, Ten Dam VH, De Craen AJM, Admiraal-Behloul F, Van Es ACGM, Palm WM, et al. Measuring longitudinal white matter changes: comparison of a visual rating scale with a volumetric measurement. *AJNR*. 2006;27:875–878.
22. Silbert LC, Nelson C, Howieson DB, Moore MM, Kaye JA. Impact of white matter hyperintensity volume progression on rate of cognitive and motor decline. *Neurology*. 2008;71:108–113.

23. Xiong YY, Mok V. Age-related white matter changes. *Journal of aging research*. 2011;2011:617927.
24. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet neurology*. 2010;9:689–701.
25. Gouw AA, Van der Flier WM, Fazekas F, Van Straaten ECW, Pantoni L, Poggesi A, et al. Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: the Leukoaraiosis and Disability study. *Stroke; a journal of cerebral circulation*. 2008;39:1414–1420.
26. Schmidt R, Enzinger C, Ropele S, Schmidt H, Fazekas F. Progression of cerebral white matter lesions: 6-year results of the Austrian Stroke Prevention Study. *Lancet*. 2003;361:2046–2048.
27. Ropele S, Seewann A, Gouw AA, Van der Flier WM, Schmidt R, Pantoni L, et al. Quantitation of brain tissue changes associated with white matter hyperintensities by diffusion-weighted and magnetization transfer imaging: the LADIS (Leukoaraiosis and Disability in the Elderly) study. *JMRI*. 2009;29:268–274.
28. Maillard P, Fletcher E, Harvey D, Carmichael O, Reed B, Mungas D, et al. White matter hyperintensity penumbra. *Stroke*. 2011;42:1917–1922.
29. Poels MMF, Steyerberg EW, Wieberdink RG, Hofman A, Koudstaal PJ, Ikram MA, et al. Assessment of cerebral small vessel disease predicts individual stroke risk. *Journal of Neurology, Neurosurgery, and Psychiatry*. 2012;83:1174–1179.

Chapter 3

Genetic Determinants of Subclinical Vascular Brain Disease

Chapter 3.1

Replication study of Chr17q25 with cerebral white matter lesion volume

Benjamin F.J. Verhaaren, Renske de Boer, Meike W. Vernooij, Fernando Rivadeneira, André G. Uitterlinden, Albert Hofman, Gabriel P. Krestin, Aad van der Lugt, Wiro J. Niessen, Monique M.B. Breteler, M. Arfan Ikram.

Stroke. 2011 Nov;42(11):3297-9.

Background and Purpose*:* Recently, the first genomewide association study on cerebral white matter lesion burden identified chr17q25 to be significantly associated with white matter lesions. We report on the first independent replication study of this genetic association.

Methods: In a population-based cohort study, we investigated the association between the 6 genomewide significant single nucleotide polymorphisms at that locus and cerebral white matter lesion volume on MRI, measured quantitatively, adjusted for age, sex, and intracranial volume. Adjustments for ApoE4 carriership and cardiovascular risk factors were evaluated separately. Finally, we performed a meta-analysis of all published data for the single most significant single nucleotide polymorphism, rs3744028.

Results: The risk alleles of all the 6 single nucleotide polymorphisms were significantly associated with white matter lesion volume with $P=1.1*10^{-3}$ for rs3744028, adjusted for age, sex, and intracranial volume. Additional adjustments only had minor influence on these associations. A meta-analysis with all published data for rs3744028 resulted in a probability value of $5.3*10^{-17}$.

Conclusions*:* This study further establishes chr17q25 as a novel genetic locus for WML volume.

INTRODUCTION

Cerebral white matter lesion (WML) burden is associated with an increased risk of stroke and dementia.[1] Unraveling the genetics of WMLs can identify potential targets for prevention and therapy. Recently, 6 single nucleotide polymorphisms mapping to 1 locus on chromosome 17q25 showed genomewide significance (P=5*10^{-8}) in the first genomewide association study on WML burden.[2] Although this association was replicated in 2 replication samples in the discovery report, further independent replication is needed to accumulate more evidence and thus strengthen the association. Here, we report on the first replication of the association of chr17q25 with WML burden after the discovery report.

Methods Study Population

This study is based on Rotterdam Study III, the second expansion of the Rotterdam Study.[3] No overlap exists between Rotterdam Study III and the samples from Rotterdam Study I and II, which were reported on in the original report.[2] In total, Rotterdam Study III comprised 3,932 persons, of whom 2,082 random persons were white and had good-quality genotype data. Of these, 1,724 had MRI data available with good-quality brain segmentations. After excluding persons with cortical infarcts (n=45) and persons with dementia (n=2), 1,677 persons were included in the analysis. Persons with cortical infarcts were excluded, because WMLs concomitant to cortical infarcts were considered to reflect a different pathology than the WMLs under study.

Genotyping

Genotyping and imputation were performed as part of a large project and are described in the online Supplemental Methods (http://stroke.aha journals.org). For this report, we extracted data on rs3744028, rs9894383, rs11869977, rs936393, rs3744017, and rs1055129. The quality of imputation of those single nucleotide polymorphisms was > 0.97. APOE genotyping was performed on coded samples as described elsewhere.[4]

MRI Measurements

MRI was performed on a 1.5-Tesla scanner (GE Healthcare) with an 8-channel head coil and included T1-weighted, proton densityweighted and fluid-attenuated inversion recovery sequences.[5] The brain tissue segmentation algorithm has been described elsewhere and is based on a k-nearest-neighbor classifier extended with WML segmentation.6 Using this classifier, we obtained volumetric measures of WMLs. Rating of infarcts was performed according to a previously described protocol.[5]

Statistical Analyses

An additive genetic model was assumed and significance of association was estimated using a 1-degree of freedom trend test relating genotype dosage, 0 to 2 copies of the minor allele, to the phenotype, that is, the log-transformed WML volume. Adjustments were for age, sex, and intracranial volume in Model I (model as in discovery study[2]) and additionally for ApoE4-carriership and cardiovascular risk factors (body mass index, diastolic and systolic blood pressure, hypertension, total cholesterol, diabetes mellitus, current smoking) in Model II. Furthermore, we repeated our analyses using WML volume as percentage of intracranial volume to directly correct for head size (Model III). We used a threshold of P=0.05 for statistical significance because our aim was to replicate the previous findings. To evaluate the possible influence of familial relationships with individuals from Rotterdam Study I and II, we performed a subanalysis after excluding persons with first-degree relatives (n=36) in Rotterdam Study I or II. Finally, we performed a meta-analysis with results from the discovery study for rs3744028 by using a Z-score pooling method.

RESULTS

Population characteristics are represented in Table 1. Table 2 shows the associations of the risk alleles of rs3744028 and the other 5 single nucleotide polymorphisms with WML volume. In Model I, presence of the risk allele of rs3744028 was associated with WML volume (difference in log-transformed mL: 0.10; 95% CI, 0.04–0.16; P=1.1*10^{-3}).

Table 1. Population characteristics

	n=1,677
Age, yr	56.1(5.3)
Female sex	55%
ApoE4-carriership	31%
BMI, kg/m^2	27.5(4.4)
Diastolic blood pressure, mmHg	82.3(10.5)
Systolic blood pressure, mmHg	131.9(18.1)
Total cholesterol, mmol/L	5.6(1.1)
Hypertension*	46%
Diabetes mellitus	7%
Current smoker	22%
WML volume, mL	3.0(3.9)

Values are means (standard deviation) or percentages
*defined as systolic blood pressure³140 mmHg or diastolic blood pressure³90 mmHg or receiving antihypertensive treatment

Table 2. Association of chr17q25 with WML volume*

SNP	*Chr:Position*	*Imputed*	R^2	*MA*	*MAF*	*Model I*		*Model II*		*Model III*	
						p-value	volume (95%CI)	p-value	volume (95%CI)	p-value	volume (95%CI)
rs3744028	17:71400267	yes	-	C	0.17	$1.1*10^{-3}$	0.10(0.04;0.16)	$3.9*10^{-3}$	0.09(0.03;0.15)	$5.8*10^{-4}$	0.11(0.05;0.17)
rs9894383	17:71377252	yes	0.90	G	0.18	$1.9*10^{-3}$	0.10(0.04;0.16)	$2.7*10^{-3}$	0.09(0.03;0.16)	$5.7*10^{-4}$	0.10(0.04;0.17)
rs11869977	17:71368977	yes	0.86	G	0.18	$2.0*10^{-3}$	0.10(0.04;0.16)	$2.8*10^{-3}$	0.09(0.03;0.16)	$9.0*10^{-4}$	0.10(0.04;0.16)
rs936393	17:71359208	yes	0.86	G	0.18	$2.3*10^{-3}$	0.09(0.03;0.15)	$3.0*10^{-3}$	0.09(0.03;0.16)	$1.1*10^{-3}$	0.10(0.04;0.16)
rs3744017	17:71383062	no	0.86	A	0.17	$6.1*10^{-4}$	0.11(0.05;0.17)	$1.5*10^{-3}$	0.10(0.04;0.16)	$2.9*10^{-4}$	0.11(0.05;0.17)
rs1055129	17:71384543	yes	0.49	G	0.28	$5.7*10^{-5}$	0.10(0.05;0.16)	$2.0*10^{-4}$	0.10(0.05;0.15)	$3.9*10^{-5}$	0.11(0.06;0.16)

Values are differences (95%CI) in WML volume per additional risk allele.
*log-transformed
Chr=chromosome. R^2= R-squared as measure of linkage disequilibrium with rs3744028. MA=minor allele=risk allele. MAF=minor allele frequency.
Model I: adjusted for age, sex and intracranial volume
Model II: as in I, additionally adjusted for apoE4-carriership and cardiovascular risk factors
Model III: WML volume expressed as percentage of intracranial volume, adjusted for age and sex

This corresponds to 9.3% of the mean WML volume. The other 5 single nucleotide polymorphisms were also significantly associated. Probability values survived Bonferroni correction. Additional adjustments (Model II) had minor influence on these associations. Expressing WML volume as a percentage of intracranial volume (Model III) slightly strengthened the associations. Finally, excluding persons with first-degree relatives in Rotterdam Study I or Rotterdam Study II did not change the results (rs3744028: 0.11; 95% CI, 0.04–0.17; P=8.9*10^{-4}). Results for other single nucleotide polymorphisms reaching P=10^{-4} in the original report are listed in online Supplemental Table I.[2] In the discovery report, rs3744028 was associated with WML volume with a P value of 4.0*10^{-15}.[2] After metaanalyzing the current data with the reported data, the P value became even more significant and reached 5.3*10^{-17}.

DISCUSSION

In this study, we successfully replicated the association between a locus on chromosome 17q25 and WML volume. To our knowledge, this is the first replication study after the discovery report. The meta-analysis of the current and previous studies yielded compelling evidence that this locus is associated with WML volume in whites. The strength of our study is the use of the same methodology, WML quantification, and analyses similar to the discovery study. In addition, our study population is relatively young compared with the populations of the discovery study, which contributes to the generalizability of the results. A limitation of the study is that although no overlap existed with the Rotterdam Study I and II cohorts reported on previously,[2] some individuals in the current study were related to persons from these cohorts. Although excluding these related persons did not influence

REFERENCES

1. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ*. 2010;341:c3666.
2. Fornage M, Debette S, Bis JC, Schmidt H, Ikram MA, Dufouil C, et al. Genome-wide association studies of cerebral white matter lesion burden: the CHARGE consortium. *Ann Neurol.* 2011;69:928 –939.
3. Hofman A, Breteler MM, van Duijn CM, Janssen HL, Krestin GP, Kuipers EJ, et al. The Rotterdam Study: 2010 objectives and design update. *Eur J Epidemiol*. 2009;24:553–572.
4. Ikram MA, Liu F, Oostra BA, Hofman A, van Duijn CM, Breteler MM. The GAB2 gene and the risk of Alzheimer's disease: replication and meta-analysis. *Biol Psychiatry*. 2009;65:995–999.
5. Vernooij MW, Ikram MA, Tanghe HL, Vincent AJ, Hofman A, Krestin GP, et al. Incidental findings on brain MRI in the general population. *N Engl J Med*. 2007;357:1821–1828.
6. de Boer R, Vrooman HA, van der Lijn F, Vernooij MW, Ikram MA, van der Lugt A, et al. White matter lesion extension to automatic brain tissue segmentation on MRI. *Neuroimage*. 2009;45:1151–1161.

Chapter 3.2

Multi-ethnic meta-analysis of genome-wide association studies on cerebral white matter lesions identifies new loci

Benjamin F.J. Verhaaren, Sudha S. Seshadri, M. Arfan Ikram,
and Myriam Fornage on behalf of the CHARGE-consortium and collaborators.

Chapter 3.3

Alzheimer's disease genes and cognition in the non-demented general population

Benjamin F.J. Verhaaren, Meike W. Vernooij, Peter J. Koudstaal, André G. Uitterlinden, Cornelia M. van Duijn, Albert Hofman, Monique M.B. Breteler, M. Arfan Ikram.

Biological Psychiatry. 2013 Mar 1;73(5):429-34.

ABSTRACT

Background: Genome-wide association studies have established 11 genes for late-onset Alzheimer's disease (AD). We investigated whether these genes jointly affect cognition in a non-demented population and also whether they improve prediction of AD.

Methods: In 5,171 non-demented people (age 45-99 years) from the population-based Rotterdam Study, we genotyped *ApoE*-ε4 and SNPs from the genes *CLU*, *PICALM, BIN1, CR1, ABCA7, MS4A6A, MS4A4E, CD2AP, EPHA1* and *CD33.* We constructed a genetic risk score by adding all the risk alleles per individual weighted by the reported effect sizes. All people underwent cognitive testing using a standardized test battery. We used linear regression to investigate the relationship between the genetic risk score and cognition, with and without *ApoE* included. In a subcohort, with more than 10 years of follow-up, we also assessed whether the risk score predicted AD.

Results: The genetic risk score was primarily associated with memory function (standardized difference in memory function (95%-CI) per SD increase in genetic risk score: (-0.05(-0.07;-0.02), $p=1.5*10^{-4}$). This association attenuated when *ApoE* was excluded from the genetic risk score (-0.03(-0.05; 0.00),p=0.047) and would not survive a multiple-testing correction. In line with this, we found that although the genetic risk score without *ApoE* was associated with the development of AD (p=0.010), it only marginally improved prediction of AD beyond age, sex, *ApoE* (AUC: 0.8159 versus 0.8148).

Conclusions: In non-demented people, there is only a marginal joint effect of AD genes on memory independent from *ApoE*. Moreover, although associated with AD, these genes jointly hardly improve prediction of AD.

INTRODUCTION

Recent meta-analyses of genome-wide association studies (GWAS) have identified 10 new genes for late onset Alzheimer's disease (AD).[1-5] In addition to *ApoE* these genes are, *CLU* (rs11136000), *PICALM* (rs3851179), *BIN1* (rs744373), *CR1* (rs3818361), *ABCA7* (rs3764650), *MS4A6A* (rs610932), *MS4A4E* (rs670139), *CD2AP (*rs9349407), *EPHA1* (rs11767557) and *CD33* (rs3865444).[1-6] Furthermore, the gene *EXOC3L2* (rs597668) has been associated with AD, though evidence for this association remains controversial.[4,7]

AD has a long preclinical phase that is characterized by accumulating pathology in the brain.[8] This pathology leads to detectable cognitive deficits already in the preclinical stages of the disease.[9,10] Given that these genes are related to AD, it remains unclear whether these genes also play a role in the preclinical phase of AD. Knowledge on this would provide essential insights into the role of these genes in the pathogenesis of AD.

The role of *ApoE*-ε4 in cognitive impairment and cognitive decline has been well established in several studies.[11-13] In contrast, few studies investigated the relation between the other AD genes and cognition in non-demented people. To our knowledge only two studies were performed that investigated GWAS genes in non-demented people, of which one study found *CR1* to be associated with cognitive decline, and the other found no robust associations.[14,15] However, these studies did not investigate *ABCA7, MS4A6A, MS4A4E, CD2AP, EPHA1* and *CD33* which are the most recently identified genes.[4,5] Moreover, these studies primarily focused on relatively old populations (mean age of 75 and older), in which the disease process of AD may be more advanced. It is unclear how AD genes relate to cognition in relatively younger populations.

Another consideration is that similar to the risk of AD, the effect sizes of individual genes on cognition may be small and therefore potentially difficult to detect. Risk scores combining individual effect sizes enable detection of more robust associations.[16]

Therefore, we studied the relation between a genetic AD risk score, based on all established risk genes, and cognition in non-demented people of the population-based Rotterdam Study and explored the associations in different age groups ranging from 45 years to 99 years. Moreover, in a subcohort we also studied whether this genetic risk score improved prediction of AD.

METHODS AND MATERIALS

Study Population

This study is based on participants from the Rotterdam Study (RS), a population-based cohort study in the Netherlands that investigates determinants of various chronic diseases

in elderly people.[17] The original study population (RS-I) consisted of 7,983 people in the general population; they were aged 55 years and older, and all residents of the Ommoord area, a suburb of Rotterdam. They were re-examined every 3 to 4 years, with the latest re-examination taking place in 2009-2011 among 2,140 remaining living people (RS-I-5).
In 2000-2001, the cohort was expanded with 3,011 people aged 55 years and over who had not yet participated (RS-II).[17] From this cohort, 2,389 people had had follow-up examinations in 2004-2005 (RS-II-2). In 2006-2008, a second expansion of 3,932 people aged 45 years and over was realized (RS-III).
The current study is based on participants from RS-I-5, RS-II-2 and RS-III; in other words, all participants from the Rotterdam Study who had examinations between 2004 and 2011. We excluded all people that were demented at baseline. In total, 1366 non-demented participants from RS-I-5, 1,737 non-demented participants from RS-II-2 and 2,068 non-demented participants from RS-III had good genotyping and cognition data.
For investigating prediction of AD we used 5,507 participants from RS-1 that were non-demented at baseline in 1990 and were followed for the development of AD until 2000.
The medical ethics committee at Erasmus University of Rotterdam, the Netherlands, approved the study, and written informed consent was obtained from all participants.

Ascertainment of dementia and AD

At study entry and all follow-up rounds all participants are screened for dementia using a 3-step protocol as follows.[18] Two brief tests of cognition (Mini-Mental State Examination [MMSE] [19] and Geriatric Mental State Schedule (GMSS) organic level [20]) were used to screen all participants. Participants who screened positive (an MMSE score of <26 or GMSS organic level of >0) underwent the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX).[21] The MMSE and the GMSS were assessed by experienced research nurses who performed these tests for all three cohorts. The CAMDEX was taken by a trained research physician. If necessary, participants who were suspected of having dementia were examined by a neuropsychologist.[18] This examination included history taking, interview, informant interview, behavioural observation and neuropsychological testing, according to conventional standards in the Netherlands. Moreover, the whole cohort is continuously monitored for onset of dementia by automated coupling of the study database with medical records of general practitioners. The diagnoses of dementia and AD were made as follows. We first diagnosed dementia in accordance with internationally accepted criteria for dementia (Diagnostic and Statistical Manual of Mental Disorders [Third Edition Revised])[22] and subsequently AD in accordance with internationally accepted criteria for AD (NINCDS-ADRDA).[23] Diagnoses were made by a single panel consisting of a neurologist, neuropsychologist, and research physician. The criteria were the same for all cohorts and remained the same over time. Participants were considered non-demented when the

criteria for dementia were not fulfilled. From the 5507 participants that were followed up for AD, 1130 screened positive in the first step (an MMSE score of < 26 or a GMSS organic level of >0) and underwent neuropsychological examination by means of the CAMDEX. From those participants, 26 required further examination by a neuropsychologist. In total, 359 participants developed AD over 10 years.

Genotyping

Genotyping was performed as part of a large project on complex diseases.[24] For Rotterdam Study I we used the Illumina HumanHap550 Duo BeadChip®; for Rotterdam Study II we used the Illumina HumanHap550 Duo BeadChip® and the Illumina Infinium II HumanHap 610 Quad Arrays®; and for Rotterdam Study III we used the Illumina Infinium II HumanHap 610 Quad Arrays®. Genotyping for Rotterdam Study I was performed in 2007, for Rotterdam Study II in 2008 and for Rotterdam Study III in 2009. For each cohort, we used the best genotyping chip that was available that time. All genotyping was done at the Human Genotyping Facility, Genetic Laboratory Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands. As detailed previously,[24] participant-specific quality controls included filters for call rate, heterozygosity, and number of Mendelian errors per individual. Single nucleotide polymorphism (SNP)-specific quality controls included filters for call rate, minor allele frequency, Hardy-Weinberg equilibrium, and differential missingness by outcome or genotype (mishap test in PLINK, http://pngu.mgh.harvard.edu/purcell/plink/). To impute to 2.5 million SNPs from NCBI build 36,[25] HapMap release #22, we used the Markov Chain Haplotyping (MaCH) package (http://www.sph.umich.edu/csg/abecasis/MACH, version 1.0.15 (Rotterdam Study I) or 1.0.16 software (for Rotterdam Study II and III). For each imputed SNP, quality of imputation was estimated as the ratio of the empirically observed dosage variance to the expected binomial dosage variance. An IBD matrix[25] was used to screen the study for latent population substructure, including cryptic relatedness. For this report we extracted data for rs11136000 (*CLU*), rs3851179 (*PICALM*), rs744373 (*BIN1*), rs3818361 (*CR1*), rs3764650 (*ABCA7*), rs610932 (*MS4A6A*), rs670139 (*MS4A4E*), rs9349407 (*CD2AP*), rs11767557 (*EPHA1*), rs3865444 (*CD33*) and rs597668 (*EXOC3L2*). *Apolipoprotein E* ε4 genotyping was performed separately as previously described.[26] From the extracted SNPs, *PICALM* (rs3851179), *BIN1* (rs744373), *CR1* (rs3818361), *ABCA7* (rs3764650), *MS4A6A* (rs610932), *MS4A4E* (rs670139), *EPHA1* (rs11767557) and *EXOC3L2* (rs597668) were genotyped and the remaining SNPs were imputed. Imputation quality was >0.95, except for rs9349407 (*CD2AP*) in RS-I (0.89), RS-II (0.90) and RS-III (0.92).

Cognitive assessment

Cognitive function was assessed with the following neuropsychological test battery: the Mini-Mental State Examination,[19] a 15-word verbal learning test (based on the Rey recall

of words,[27] the Stroop test,[28] the Letter-Digit Substitution Task (LDST)[29] and a word fluency test (animal categories).[30] All examinations were performed by the same research team at a single study site and were exactly the same for all cohorts. For each cognitive test we generated Z-scores (individual test score minus mean test score divided by the standard deviation). To obtain more robust measures, we constructed compound scores for memory, executive function, information processing speed, and global cognition. The choice of these compound scores was corroborated by a principal component analysis. The Z-scores for the Stroop tasks were inverted for use in these compound scores while higher scores on the Stroop task indicate a worse performance while higher scores on all other tests indicate a better cognitive function. 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. Executive function was constructed by averaging the Z-scores for the Stroop interference subtask, the LDST (number of correct digits in 1 minute), and the word fluency test (number of animals in 1 minute). Information processing speed was the average of the Z-scores for the Stroop reading and Stroop color naming test and the LDST. For global cognitive function, we used the average of the Z-scores of the Stroop task (average of all 3 subtasks), the LDST, the word fluency test, and the immediate and delayed recall of the 15-word verbal learning test. The participants' attained level of education was assessed by interview according to the standard classification of education.[31] In our analysis, we combined the 4 highest levels into 1 category, thus obtaining 4 levels: (1) primary education; (2) low-level vocational training; (3) medium level secondary education; and (4) medium-level vocational training to university level.

Statistical analyses

We constructed a genetic risk score, by adding up all the risk alleles per individual weighted by their log-transformed, reported effect size on AlzGene[32] for the association with AD. A higher genetic risk score corresponds to a larger number of risk alleles and thus a higher risk for AD. For the main analyses we excluded *EXOC3L2* because of its reported controversy.[4,7] We investigated the relationship between the genetic risk score (per standard deviation increase as well as in quintiles) and the compound scores of cognitive function using linear regression models, adjusted for age, sex, level of education and cohort. We adjusted for cohort to take into account any potential differences across cohorts, e.g. in genotyping platforms. We first investigated the associations in the total population, and subsequently in age strata. For this, we chose age strata of 45-60 years, 60-70 years and > 70 years to contain roughly equal number of people per stratum. To test for potential age-dependent or sex-specific effects of the risk score, the addition of the interaction terms [genetic risk score x age stratum] and [genetic risk score x sex] was evaluated. For each stratum we had more than 99 % statistical power to find small effect sizes (Cohen's $f^2 = 0.02$) with $\alpha = 0.05$.[33]

Analyses were repeated using a risk score in which *ApoE*-ε4 was not incorporated. Next, we also evaluated the influence of *ApoE*-ε4, by taking it as a covariate in the statistical model. Also, we studied whether *ApoE*-ε4 interacted with the *ApoE*-ε4-excluded genetic risk score by adding an interaction term [genetic risk score x *ApoE*-ε4] to the model. Furthermore, we additionally applied a Bonferroni correction for (4 domains x 4 age groups =) 16 tests to all p-values.

In addition, we used Cox proportional hazard models to study whether the genetic risk score without *ApoE* was associated with the development of AD in RS-I from 1990 to 2000. Moreover, we also investigated whether the risk score improved prediction of AD beyond age, sex, and *ApoE*. Hereto, we used logistic regression models to calculate the area under the receiver operating curve (AUC) at ten years of follow-up for three different prediction models: 1) age and sex, 2) age, sex and *ApoE*-ε4 carriership, 3) age, sex, *ApoE*-ε4 carriership and the risk score.

Finally, we repeated all analyses after adding *EXOC3L2* to the risk scores.

RESULTS

The characteristics of the Alzheimer's disease (AD) genes in the study population can be found in Table 1. Table 2 shows the association of the genetic risk scores with cognition. We found that the genetic risk score was associated with global cognition (difference in Z-score (95% confidence interval) per SD increase in genetic risk score: -0.03 (-0.05; -0.01), p=0.001), which was primarily driven by memory function (-0.05 (-0.07; -0.02), $p=1.5*10^{-4}$). Stratification of the population in age groups of 45-60 years, 60-70 years and 70-99 years, revealed that across age strata a trend of increasing effect sizes was evident, although the associations were primarily present in people older than 70 years (Table 2). In line with this, addition of the interaction term [genetic risk score x age stratum] was strongly significant ($p=8.9*10^{-4}$ for global cognition, $p=1.2*10^{-3}$ for memory function). Addition of an interaction term [genetic risk score x sex] was not significant for any cognitive domain (p>0.15).

After exclusion of *ApoE*-ε4 from the risk score we found that the associations attenuated (e.g., -0.02 (-0.03; 0.00), p= 0.087 for global cognition and -0.03 (-0.05; 0.00), p=0.047 for memory function) (Table 2). We note that though nominally significant, the latter p-value would not survive correction for multiple-testing. When analyzed per age-stratum, the risk score without *ApoE* was not significantly associated with cognition, although a negative effect on cognition was noticeable. This effect did not appear stronger in the higher age strata. In line with this, addition of the interaction term [genetic risk score x age stratum] was no longer significant (p=0.699 for global cognition, p=0.986 for memory function). Addition of an interaction term [genetic risk score x sex] was not statistically significant

Table 1. Characteristics of the Alzheimer's disease genes in the study population

Gene	*SNP*	*Chromosome*	*Imputed*	*RA*	*RAF*	*Reported Odds Ratio**
ApoE	-	19	no	ε4	0.20	3.685
CLU	rs11136000	8	yes	C	0.60	1.138
PICALM	rs3851179	11	no	C	0.63	1.138
BIN1	rs744373	2	no	G	0.30	1.166
CR1	rs3818361	1	no	A	0.19	1.174
ABCA7	rs3764650	19	no	G	0.08	1.229
MS4A6A	rs610932	11	no	G	0.57	1.106
MS4A4E	rs670139	11	no	T	0.42	1.079
CD2AP	rs9349407		yes	C	0.27	1.117
EPHA1	rs11767557	7	no	T	0.80	1.124
CD33	rs3865444	19	yes	C	0.67	1.120
EXOC3L2	rs597668	19	no	C	0.17	1.170

*for late onset Alzheimer's disease based on AlzGene (32). Abbreviations: SNP = single nucleotide polymorphism, RA = risk allele, RAF = risk allele frequency.

Table 3. Age, sex, ***ApoE***-ε4-carriership, the genetic risk score without ***ApoE*** and their relation with Alzheimer's disease for three different models

	HR (95% CI)	**AUC (95% CI)**
Model I:	-	0.7934 (0.7728; 0.8140)
Age	1.15 (1.13; 1.16)	
Sex	1.38 (1.09; 1.74)	
Model II:	-	0.8148 (0.7959; 0.8337)
Age	1.15 (1.14; 1.17)	
Sex	1.40 (1.10; 1.77)	
ApoE-ε4-carriership	2.60 (2.11; 3.20)	
Model III:	-	0.8159 (0.7970; 0.8348)
Age	1.15 (1.14; 1.17)	
Sex	1.39 (1.10; 1.77)	
ApoE-ε4-carriership	2.61 (2.12; 3.22)	
Genetic risk score without *ApoE*	1.14 (1.03; 1.27)*	

***** per standard deviation increase. Abbreviations: HR = hazard ratio for Alzheimer's disease, AUC = Area Under the Curve at 10 years of follow-up, CI = confidence interval.

Table 2. Associations between the genetic risk scores and cognition

	Global cognition			Memory function			Executive function			Information processing speed		
All ages (n = 5,171)												
Genetic risk score 1	-0.03	(-0.05; -0.01)	p=0.001	-0.05	(-0.07; -0.02)	$p=1.5*10^{-4}$	-0.02	(-0.04; 0.00)	p=0.019	-0.02	(-0.04; 0.00)	p=0.055
Genetic risk score 2	-0.02	(-0.03; 0.00)	p=0.087	-0.03	(-0.05; 0.00)	p=0.047	-0.02	(-0.03; 0.00)	p=0.100	-0.01	(-0.03; 0.01)	p=0.402
Age strata												
45-60 (n = 1,662)												
Genetic risk score 1	0.01	(-0.02; 0.04)	p=0.538	0.01	(-0.04; 0.05)	p=0.793	0.01	(-0.02; 0.04)	p=0.529	0.01	(-0.02; 0.04)	p=0.488
Genetic risk score 2	-0.02	(-0.05; 0.01)	p=0.194	-0.02	(-0.06; 0.03)	p=0.516	-0.02	(-0.05; 0.01)	p=0.119	-0.02	(-0.05; 0.01)	p=0.212
60-70 (n = 1,644)												
Genetic risk score 1	-0.02	(-0.05; 0.01)	p=0.195	-0.05	(-0.09; -0.01)	p=0.023	0.00	(-0.03; 0.02)	p=0.768	-0.01	(-0.04; 0.02)	p=0.531
Genetic risk score 2	-0.01	(-0.04; 0.02)	p=0.449	-0.04	(-0.09; 0.00)	p=0.061	0.00	(-0.03; 0.03)	p=0.901	0.01	(-0.03; 0.04)	p=0.717
70-99 (n = 1,865)												
Genetic risk score 1	-0.08	(-0.12; -0.05)	$p=6.7*10^{-7}$	-0.10	(-0.14; -0.06)	$p=1.0*10^{-6}$	-0.07	(-0.11; -0.04)	$p=8.7*10^{-5}$	-0.06	(-0.10; -0.02)	p=0.003
Genetic risk score 2	-0.02	(-0.05; 0.02)	p=0.339	-0.02	(-0.06; 0.02)	p=0.415	-0.02	(-0.05; 0.01)	p=0.244	-0.01	(-0.05; 0.03)	p=0.544

Values are differences in Z-scores of cognition (95% confidence intervals) per SD deviation increase of the genetic risk score, adjusted for age, sex, level of education and cohort.
Genetic risk score 1 = full genetic risk score (including *CLU, PICALM, BIN1, CR1, ABCA7, MS4A6A, MS4A4E, CD2AP, EPHA1 CD33* and *ApoE).*
Genetic risk score 2 = genetic risk score 1 without *ApoE*.

either (p>0.15). We also did not find any interaction between the presence of *ApoE*-ε4 and the *ApoE*-excluded genetic risk score (p=0.285 for global cognition, p=0.133 for memory function). Adjusting the risk score for *ApoE*-ε4 did not have any influence on the associations. After 10 years follow-up, 359 persons of the 5,507 developed AD. Table 3 shows that the genetic risk score without *ApoE* was associated with the development of AD. We found a hazard ratio per standard deviation increase in risk score of 1.14 (1.03; 1.27) with p-value 0.010. In contrast, the genetic risk score did not predict AD beyond age, sex and *ApoE*-ε4-carriership with the area under the curve only marginally improving from 0.8148 to 0.8159. Finally, addition of *EXOC3L2* to the genetic risk scores revealed similar results.

DISCUSSION

In this population-based study of over 5,000 non-demented middle-aged and elderly, we found only a marginal joint effect of established AD genes on memory independent from *ApoE*. Moreover, we found that these genes, although associated with AD, jointly only marginally improve prediction of AD.

Strengths of this study are its large sample size, population-based setting and the wide age range. Moreover, we assessed cognitive function covering three cognitive domains. A limitation of this study is the absence of longitudinal assessment of cognitive performance and therefore no information on cognitive decline. Another consideration is that non-demented persons with a high genetic risk score may not have participated because they already have subclinical AD. We believe that – if present – this would not negatively affect our study as our main purpose was to investigate the influence of genes on cognition in a *non-demented* population. Finally, we note that the RS-I cohort used for prediction of AD was also among the discovery cohorts of the AD genes. [3,5] However, removal of any potential bias would only even further decrease the predictive accuracy of these genes.To our knowledge we are the first to study the relationship of a genetic risk score for AD based on the most recent set of genome-wide significant SNPs[1-5] with cognition in a non-demented population. We found that, though the risk score related to poorer cognition, this was mainly driven by detrimental effects of *ApoE*-ε4. The relation between *ApoE*-ε4 and cognition has been well established in several studies as well as their age-dependent relationship.[11,13,34] We further corroborate this by showing that of all known genetic influences on cognition *ApoE*-ε4 is by far the strongest and even exceeds the joint effect of the remaining known genetic genes, and that this relationship is mainly present in people older than 70 years. So far, two studies have reported on the relationship between novel GWAS genes and cognition. Hamilton et al investigated the relationship between *CLU*, *PICALM*, *EXOC3L2*, *CR1* and *BIN1*, and cognitive performance in 505 non-demented people of mean age 79, and found no robust

associations.[14] Chibnik et al investigated the relationship between *CLU*, *PICALM*, *EXOC3L2*, *CR1* and cognitive decline over 7.8 and 4.3 years of follow up in two non-demented cohorts of 791 and 875 persons (mean age 76 and 81 years at baseline), and found CR1 to be significantly related with cognitive decline.[15] In addition they found that *CR1* was related to AD-neuropathology.[15] Nevertheless, in our study of more than 5000 people we did not find any association for *CR1* despite the fact that our study also comprised individuals of old age. A likely explanation for this is that cognitive decline at old age reflects incident AD better than cognitive function measured only once. This is supported by Chibnik et al who did not find CR1 to be associated with cognitive function at baseline.[15]

It is interesting that the AD genes apart from *ApoE* are not markedly related to cognition in our study and that even the joint effect of these genes, although associated with AD, hardly improved prediction of AD. There are several explanations for this finding. First, this suggests that the influence of these genes on cognition is very subtle. Secondly, it could also be that there are competing environmental risks that mask the role of these genes in the pathogenesis of AD. Thirdly, medication use or physical and mental illnesses might have hindered the detection of an association. Another explanation is that the risk alleles of these genes may not primarily cause cognitive decline themselves, but mainly interact with environmental risk factors, e.g., cardiovascular disease. It could also be argued that these genes do not have any causal relation with the onset of cognitive decline, but merely serve as accelerators of cognitive decline. Evidence for this comes from a recent report that showed that plasma levels of clusterine (the protein coded by *CLU*) are only associated with the prevalence and severity of AD, but not with the incidence of AD.[35]

Finally, another consideration is that the SNPs used for our study are likely not the causal variants, but merely in linkage disequilibrium with them. Consequently, we foresee that future studies employing sequencing data have the potential to find stronger associations and will improve the prediction of AD.

In conclusion, we found only a marginal joint effect of established AD genes on memory independent from *ApoE.* Moreover, despite their relationship with AD, these genes hardly improve prediction of AD beyond age, sex and *ApoE*. More studies are needed to further understand the role of these genes in the etiology of Alzheimer disease. Such studies for example can focus on biological pathways, in which these genes have been implicated, such as inflammatory or lipid pathways.[5] Furthermore, discovery of additional AD genes in coming years and the further pin-pointing of the exact causal polymorphism by sequencing may reveal a more pronounced joint effect of all these AD genes on cognition and may lead to better prediction of AD, especially when compared to the current set of 11 genes.

REFERENCES

1. Lambert JC, Heath S, Even G, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet.* Oct 2009;41(10):1094-1099.
2. Harold D, Abraham R, Hollingworth P, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet.* Oct 2009;41(10):1088-1093.
3. Seshadri S, Fitzpatrick AL, Ikram MA, et al. Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA.* May 12 2010;303(18):1832-1840.
4. Naj AC, Jun G, Beecham GW, et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet.* May 2011;43(5):436-441.
5. Hollingworth P, Harold D, Sims R, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet.* May 2011;43(5):429-435.
6. Bertram L, Lange C, Mullin K, et al. Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. *Am J Hum Genet.* Nov 2008;83(5):623-632.
7. Carrasquillo MM, Belbin O, Hunter TA, et al. Replication of BIN1 association with Alzheimer's disease and evaluation of genetic interactions. *J Alzheimers Dis.* 2011;24(4):751-758.
8. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* May 2011;7(3):280-292.
9. La Rue A, Jarvik LF. Cognitive function and prediction of dementia in old age. *Int J Aging Hum Dev.* 1987;25(2):79-89.
10. Elias MF, Beiser A, Wolf PA, Au R, White RF, D'Agostino RB. The preclinical phase of alzheimer disease: A 22-year prospective study of the Framingham Cohort. *Arch Neurol.* Jun 2000;57(6):808-813.
11. Caselli RJ, Dueck AC, Osborne D, et al. Longitudinal modeling of age-related memory decline and the APOE epsilon4 effect. *N Engl J Med.* Jul 16 2009;361(3):255-263.
12. Small BJ, Rosnick CB, Fratiglioni L, Backman L. Apolipoprotein E and cognitive performance: a meta-analysis. *Psychol Aging.* Dec 2004;19(4):592-600.
13. Anstey K, Christensen H. Education, activity, health, blood pressure and apolipoprotein E as predictors of cognitive change in old age: a review. *Gerontology.* May-Jun 2000;46(3):163-177.
14. Hamilton G, Harris SE, Davies G, et al. Alzheimer's disease genes are associated with measures of cognitive ageing in the lothian birth cohorts of 1921 and 1936. *Int J Alzheimers Dis.* 2011;2011:505984.
15. Chibnik LB, Shulman JM, Leurgans SE, et al. CR1 is associated with amyloid plaque burden and age-related cognitive decline. *Ann Neurol.* Mar 2011;69(3):560-569.
16. International Schizophrenia C, Purcell SM, Wray NR, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature.* Aug 6 2009;460(7256):748-752.
17. Hofman A, van Duijn CM, Franco OH, et al. The Rotterdam Study: 2012 objectives and design update. *Eur J Epidemiol.* Aug 30 2011.
18. Ott A, Breteler MM, van Harskamp F, Stijnen T, Hofman A. Incidence and risk of dementia. The Rotterdam Study. *Am J Epidemiol.* Mar 15 1998;147(6):574-580.
19. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* Nov 1975;12(3):189-198.
20. Copeland JR, Kelleher MJ, Kellett JM, et al. A semi-structured clinical interview for the assessment of diagnosis and mental state in the elderly: the Geriatric Mental State Schedule. I. Development and reliability. *Psychol Med.* Aug 1976;6(3):439-449.
21. Roth M, Tym E, Mountjoy CQ, et al. CAMDEX. A standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. *Br J Psychiatry.* Dec 1986;149:698-709.
22. *Diagnostic and Statistical Manual of Mental Disorders. American Psychiatric Association.* 3rd, rev. ed. Washington, DC1987.

23. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology.* Jul 1984;34(7):939-944.
24. Ikram MA, Seshadri S, Bis JC, et al. Genomewide association studies of stroke. *N Engl J Med.* Apr 23 2009;360(17):1718-1728.
25. Richards JB, Rivadeneira F, Inouye M, et al. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. *Lancet.* May 3 2008;371(9623):1505-1512.
26. Ikram MA, Liu F, Oostra BA, Hofman A, van Duijn CM, Breteler MM. The GAB2 gene and the risk of Alzheimer's disease: replication and meta-analysis. *Biol Psychiatry.* Jun 1 2009;65(11):995-999.
27. Bleecker ML, Bolla-Wilson K, Agnew J, Meyers DA. Age-related sex differences in verbal memory. *J Clin Psychol.* May 1988;44(3):403-411.
28. Golden CJ. Identification of brain disorders by the Stroop Color and Word Test. *J Clin Psychol.* Jul 1976;32(3):654-658.
29. Lezak MD HD, Loring DW. *Neuropsychological assesment.* New York, NY: Oxford University Press; 2004.
30. Welsh KA, Butters N, Mohs RC, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part V. A normative study of the neuropsychological battery. *Neurology.* Apr 1994;44(4):609-614.
31. Vernooij MW, Ikram MA, Vrooman HA, et al. White matter microstructural integrity and cognitive function in a general elderly population. *Arch Gen Psychiatry.* May 2009;66(5):545-553.
32. Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. *Nat Genet.* Jan 2007;39(1):17-23.
33. Cohen J, Cohen, P., West, S.G., and Aiken, L.S. . *Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences.* 3rd ed. Mahwah, NJ, USA: Lawrence Earlbaum Associates; 2003.
34. Jochemsen HM, Muller M, van der Graaf Y, Geerlings MI. APOE epsilon4 differentially influences change in memory performance depending on age. The SMART-MR study. *Neurobiol Aging.* Sep 9 2011.
35. Schrijvers EM, Koudstaal PJ, Hofman A, Breteler MM. Plasma clusterin and the risk of Alzheimer disease. *JAMA.* Apr 6 2011;305(13):1322-1326.

Chapter 3.4

The effect of the genetics of blood pressure on retinal and cerebral circulation in the general population

Benjamin F.J. Verhaaren, Meike W. Vernooij, Caroline C.W. Klaver, André G. Uitterlinden, Cornelia M. van Duijn, CHARGE Blood Pressure working group; Albert V. Smith, Vilmundur Gudnason, Mary F. Cotch, Mark A. van Buchem, Albert Hofman, Aad van der Lugt, Lenore J. Launer, M. Arfan Ikram.

Chapter 4

Modifiable Determinants of Subclinical Vascular Brain Disease

Chapter 4.1

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

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

Neurology. 2012 May 8;78(19):1456-63.

ABSTRACT

Objective: To investigate whether dementia incidence has changed over the last 2 decades.

Methods: We compared dementia incidence in 2 independent subcohorts of persons aged 60–90 years from the Rotterdam Study, a population-based cohort study. The first subcohort started in 1990 (n = 5,727), the second in 2000 (n = 1,769). Participants were dementia-free at baseline and followed for at maximum 5 years. We calculated age-adjusted dementia incidence rates for the 2 subcohorts in total, in 10-year age strata, and for men and women separately. We also compared mortality rates, differences in prevalence of vascular risk factors, and medication use. Finally, we compared brain volumes and the extent of cerebral small vessel disease in participants who underwent brain imaging 5 years after the baseline examinations.

Results: In the 1990 subcohort (25,696 person-years), 286 persons developed dementia, and in the 2000 subcohort (8,384 person-years), 49 persons. Age-adjusted dementia incidence rates were consistently, yet nonsignificantly, lower in the 2000 subcohort in all strata, reaching borderline significance in the overall analysis (incidence rate ratio 0.75, 95% confidence interval [CI] 0.56–1.02). Mortality rates were also lower in the 2000 subcohort (rate ratio 0.63, 95% CI0.52–0.77). The prevalence of hypertension and obesity significantly increased between 1990 and 2000. This was paralleled by a strong increase in use of antithrombotics and lipid-loweringdrugs. Participants in 2005–2006 had larger total brain volumes ($p < 0.001$) and less cerebral small vessel disease (although nonsignificant in men) than participants in 1995–1996.

Conclusions: Although the differences in dementia incidence were nonsignificant, our study suggests that dementia incidence has decreased between 1990 and 2005.

INTRODUCTION

The prevalence of dementia is increasing. Due to the aging of the population, the number of persons living with dementia worldwide is expected to double every 20 years, with an expected number of 81 million people with dementia in 2040.[1] Future projections typically assume stable incidence rates of dementia, and do not take better prevention into account, which could lower the incidence and thereby cause a smaller rise in dementia prevalence.[2,3] Studies investigating trends in dementia incidence have reported different results. Some have reported no change in incidence rates.[4–7] Two studies that based the diagnosis of dementia solely on medical records reported increasing incidence rates, which will be at least in part a reflection of improved diagnostic procedures for dementia and an increase in case identification and diagnosispopularity.[8,9] A recent article reported decreasing incidence rates between 1985 and 1994.[10] For stroke, incidence rates have declined over the past 4 decades in high-income countries, presumably due to the implementation of preventive treatments and reduction in risk factors at the population level.[11,12] Since vascular risk factors increase the risk for dementia,[13] we hypothesized that incidence rates of dementia could have likewise declined. To investigate whether dementia incidence changed over the last 2 decades, we compared the age-specific incidence rates of dementia and mortality in a cohort of elderly persons that started in 1990 with a cohort that started in 2000, both from the Rotterdam Study, a large population-based cohort study from theNetherlands.1[4] Furthermore, we compared brain volumes and the presence of cerebral small vessel disease between participants of both subcohorts who underwent a brain MRI 5 years after study entry.

METHODS

Study population

The Rotterdam Study started in 1990 and is conducted among all inhabitants aged 55years and older of Ommoord, a district of Rotterdam, the Netherlands.[14] The study was extended in 2000 with a new subcohort of persons who had become 55 years of age or moved into the study district since the start of the study. Follow-up examinations were repeated every 3 to 4 years. In addition, through linkage with records of general practitioners and the municipality, the total cohort was continuously monitored for morbidity and mortality. Details of the study have been described elsewhere.[14] For the current study, we included all participants who were between 60 and 90 years, because of limited numbers of incident dementia under age 60, and limited numbers of participants older than 90. Of the 6,485 participants between 60 and 90 in the 1990 subcohort, 6,100 participants were screened for dementia and prevalent

dementia was diagnosed in 373 participants. In total, 5,727 participants were included in the analyses. In the 2000 subcohort, of 1,992 participants between 60 and 90 years, 1,783 were screened for dementia and prevalent dementia was diagnosed in 14 participants. In total, 1,769 participants were included in the analyses.

Standard protocol approvals, registrations, and patient consents

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

Dementia case finding

Participants were screened for dementia at baseline and follow-up visits using a 3-step protocol.[15] Two brief tests of cognition (Mini-Mental State Examination [MMSE][16] and Geriatric Mental State schedule [GMS][17] organic level) were used to screen all subjects. Screen-positives (MMSE score <26 or GMS organic level >0) underwent the Cambridge examination for mental disorders of the elderly.[18] Subjects who were suspected of having dementia were, if necessary, examined by a neuropsychologist. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.[15] The diagnosis of dementia was made in accordance with internationally accepted criteria (*DSM-III-R*)[19] by a panel of a neurologist, neuropsychologist, and research physician. The follow-up with regard to dementia diagnosis was virtually complete until January 1, 2007.

Baseline characteristics

Educational level was dichotomized into primary education (with or without an unfinished higher education) vs lower vocational to university education. Smoking habits were categorized as current, former, and never cigarette smoking. History of stroke or myocardial infarction at baseline was verified by reviewing medical records. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in a sitting position. Hypertension was defined as a blood pressure >=160/95 or use of antihypertensive medication, prescribed for the indication of hypertension. The waist circumference was measured in centimeters. Diabetes mellitus was defined as a self-reported history of diabetes, a random nonfasting or postload serum glucose level >=11.1 mmol/L (1990 subcohort), or a random fasting serum glucose level >=7.0 mmol/L (2000 subcohort).

Brain imaging

Brain imaging was performed as part of the Rotterdam Scan Study.[20,21] In 1995–1996, a

random subset of the 1990 subcohort was invited to undergo brain imaging on a1.5 T MRI scanner.20 From 2005 onwards, MRI imaging on a1.5 T MRI scanner is routinely performed as part of the core examinations of the Rotterdam Study.[21] Details of the imaging protocol, of the image processing for assessment of brain atrophy and presence of white matter lesions (WML), and of the rating of lacunar and cortical infarcts are given in appendix e-1 on the *Neurology®* Web site at www.neurology.org and have been describedelsewhere.[20,21] In total, we had brain imaging data for 487 participants from the 1990 subcohort, and for 864 participants of the 2000 subcohort. All participants were between 60 and 90 years and free of dementia at the time of MRI scanning, which occurred 5 to 6 years after study entry.

Statistical analyses

Baseline characteristics were compared between 1990 and 2000 per 10-year age strata, for men and women separately. Differences between the subcohorts were assessed using linear regression for continuous variables and logistic regression for dichotomous variables, adjusted for age. Fisher exact test was used for dichotomous variables if a percentage was zero. For the current study, participants contributed person-years for a maximum of 5 years after baseline. For the incidence of dementia, follow-up time was censored at date of dementia diagnosis, date of death, date of reaching age 90, or 5 years after baseline, whatever came first. Five-year follow-up was complete for more than 99% for both subcohorts. Dementia incidence rates, mortality rates, and incidence rate ratios (IRR) were calculated using Poisson regression models for the 2 subcohorts in total, in 10-year age strata, and for men and women separately. All analyses were adjusted for age, and an additional adjustment was made for age squared, to make sure the effect of age was adequately adjusted for. Intracranial volume, total brain volume, and volume of WML on MRI were compared between the subcohorts in total, in 10-year age strata, and for men and women separately, using analysis of variance, adjusting for age. Total brain volume and WML were expressed as percentages of intracranial volume. Because of a non-normal distribution, WML were natural log transformed to compare the differences. Odds ratios for the presence of cortical and lacunar infarcts were calculated using logistic regression models, adjusted for age.

RESULTS

Baseline characteristics in strata of age and sex are presented in table 1.

Due to the design of the study, the age distribution was different in the 1990 subcohort compared to the 2000 subcohort. The distribution in the 2000 subcohort is more skewed toward younger participants who became eligible for participation only after the start of

the first subcohort. In the 60–69 age stratum, both men and women were significantly younger in the 2000 subcohort. The 2000 subcohort was higher educated across all strata. Participants in the 2000 subcohort had higher blood pressure and more often hypertension, a higher body mass index and larger waist circumference, and had smoked more often before but were smoking less at present. More participants in the 2000 subcohort had diabetes and used antidiabetic medication, although the differences were mostly nonsignificant. The use of antithrombotics was 3- to 6-fold higher in the 2000 subcohort compared to the use in 1990 ($p < 0.001$ across all strata), primarily due to higher use of platelet aggregation inhibitors. Also, the use of lipid-lowering drugs was much higher in the 2000 cohort ($p < 0.001$ across all strata, except men in the highest age stratum).In the 1990 subcohort, after 25,696 person-years,286 incident dementia cases had occurred, and in the 2000 subcohort, 49 incident dementia cases had occurred after 8,384 person-years. Age-adjusted dementia incidence rates and IRR are shown in table 2.The incidence of dementia was 25% lower in the 2000 subcohort compared to the 1990 subcohort, reaching borderline significance (IRR 0.75, 95% confidence interval [CI] 0.56 –1.02, $p = 0.06$). The dementia incidence was nonsignificantly lower in the 2000 subcohort across all age strata. For men, the difference was largest in the 70–79 age stratum (IRR0.48, 95% CI 0.21–1.11), while there was no difference in dementia incidence in the highest age stratum from 80 to 89 years. For women, the estimated reduction in dementia incidence increased from 9%in the lowest age stratum to 23% in the highest stratum. Further adjustment for age squared slightly changed the incidence rates, but not the IRR. In the 1990 subcohort, 782 persons died, 709without having a diagnosis of dementia. In the 2000subcohort, 119 persons died, 112 without dementia. Age-adjusted mortality rates and rate ratios are shown in table 3. Overall, the age-adjusted mortality rate was 37% lower in the 2000 subcohort compared to the 1990 subcohort (rate ratio 0.63, 95% CI0.52– 0.77, $p < 0.001$). For both men and women, the mortality rates were lower in the 2000 subcohort, although this was not significant in every age stratum. In men, the difference was most pronounced in the highest age stratum, where the mortality rate was64% lower in the 2000 subcohort. In women, the difference was 68% in the youngest age stratum, 21% in the 70–79 stratum, and 43% in the highest age stratum. Further adjustment for age squared did not change the results. Total brain volume was higher in 2005–2006 compared to 1995–1996 for both men and women in all age strata (p values <=0.007) (table 4). The WML volume was consistently lower in 2005–2006, though not always significantly so. In 1995–1996, cortical infarcts were present in 24 participants (5%) vs 19 participants (2%) in 2005–2006 (OR 2005–2006 vs 1995–1996: 0.75, 95% CI 0.38 –1.49). We had insufficient power to test differences in strata. Lacunar infarcts were present in 112 participants (23%) in 1995–1996 and in 59 participants (7%) in2005–2006 (OR 0.43, 95% CI 0.30–0.63). The difference was more pronounced in women (OR 0.19, 95% CI 0.10–0.34) than in men (OR 0.81, 95% CI 0.49 –1.35).

Table 1. Baseline characteristics in strata of age and sex

		Age category					
		60-69		70-79		80-89	
	Subcohort	Men	Women	Men	Women	Men	Women
Number	1990	1241	1532	824	1259	248	623
	2000	553	636	194	255	50	101
Age (years)	1990	64.9 ± 2.8	64.9 ± 2.9	74.4 ± 2.9	74.6 ± 2.8	83.8 ± 2.7	84.1 ± 2.7
	2000	63.6 ± 2.8	63.1 ± 2.5	74.2 ± 2.8	74.7 ± 2.7	83.6 ± 2.4	83.6 ± 2.7
	P-value	<0.001	<0.001	0.45	0.46	0.57	0.06
Only primary education	1990	243 (20%)	576 (38%)	267 (33%)	623 (51%)	111 (48%)	395 (70%)
	2000	86 (16%)	164 (27%)	36 (19%)	99 (39%)	12 (24%)	40 (40%)
	P-value	0.23	<0.001	<0.001	<0.001	0.003	<0.001
Systolic blood pressure (mmHg)	1990	138 ± 21	136 ± 21	143 ± 23	145 ± 21	142 ± 23	151 ± 23
	2000	143 ± 21	141 ± 19	151 ± 23	155 ± 24	150 ± 24	163 ± 22
	P-value	<0.001	<0.001	<0.001	<0.001	0.03	<0.001
Diastolic blood pressure (mmHg)	1990	76 ± 11	74 ± 11	73 ± 12	73 ± 11	71 ± 12	73 ± 13
	2000	81 ± 10	78 ± 10	79 ± 11	78 ± 11	77 ± 13	77 ± 12
	P-value	<0.001	<0.001	<0.001	<0.001	0.002	0.01
Hypertension >=160/95	1990	375 (31%)	504 (33%)	287 (37%)	564 (47%)	75 (35%)	293 (53%)
	2000	185 (36%)	194 (32%)	98 (51%)	139 (56%)	25 (51%)	70 (70%)
	P-value	0.02	0.46	<0.001	0.007	0.03	0.002
Body mass index (kg/m^2)	1990	25.8 ± 2.9	26.8 ± 4.0	25.7 ± 3.0	27.0 ± 4.1	24.7 ± 3.4	27.1 ± 4.2
	2000	27.0 ± 3.4	27.5 ± 4.5	26.5 ± 3.1	27.7 ± 4.4	25.5 ± 2.9	27.0 ± 3.9
	P-value	<0.001	<0.001	0.001	0.02	0.160	0.728
Waist circumference (cm)	1990	94.1 ± 9.1	87.1 ± 11.3	95.1 ± 9.7	88.8 ± 11.5	95.0 ± 10.7	92.1 ± 12.0
	2000	98.6 ± 9.8	89.5 ± 11.5	99.8 ± 9.3	91.7 ± 11.5	98.4 ± 8.4	91.8 ± 10.8
	P-value	<0.001	<0.001	<0.001	0.001	0.05	0.83
Smoking: Current	1990	322 (27%)	348 (23%)	167 (22%)	155 (13%)	54 (26%)	30 (5%)
	2000	119 (23%)	125 (20%)	24 (13%)	35 (14%)	1 (2%)	8 (8%)
	P-value	0.02	0.07	0.004	0.64	0.006	0.34
Smoking: Never	1990	100 (8%)	670 (45%)	103 (13%)	738 (61%)	40 (19%)	436 (75%)
	2000	94 (18%)	227 (37%)	22 (11%)	109 (43%)	7 (14%)	58 (57%)
	P-value	<0.001	0.01	0.49	<0.001	0.42	0.001
Diabetes	1990	121 (10%)	126 (8%)	105 (13%)	152 (12%)	38 (16%)	118 (20%)
	2000	55 (10%)	58 (9%)	35 (18%)	37 (15%)	9 (18%)	8 (8%)
	P-value	0.42	0.08	0.06	0.31	0.62	0.007
Myocardial infarction	1990	138 (11%)	34 (2%)	117 (15%)	56 (5%)	23 (10%)	39 (7%)
	2000	36 (7%)	13 (2%)	26 (13%)	6 (2%)	6 (12%)	7 (7%)
	P-value	0.01	0.52	0.69	0.11	0.63	0.88
Stroke	1990	25 (2%)	18 (1%)	40 (5%)	26 (2%)	22 (9%)	37 (6%)
	2000	18 (3%)	10 (2%)	12 (6%)	6 (2%)	4 (8%)	7 (7%)
	P-value	0.10	0.20	0.44	0.79	0.84	0.66
Blood pressure lowering drugs	1990	348 (28%)	440 (29%)	279 (34%)	515 (41%)	92 (37%)	324 (52%)
	2000	138 (26%)	152 (24%)	76 (39%)	123 (48%)	22 (44%)	43 (43%)
	P-value	0.62	0.40	0.15	0.04	0.37	0.08
Antidiabetic therapy	1990	49 (4%)	47 (3%)	49 (6%)	82 (7%)	12 (5%)	49 (8%)
	2000	24 (5%)	22 (4%)	21 (11%)	20 (8%)	3 (6%)	3 (3%)
	P-value	0.30	0.13	0.02	0.46	0.75	0.08
Antithrombotics	1990	81 (7%)	38 (2%)	90 (11%)	45 (4%)	26 (11%)	39 (6%)
	2000	116 (22%)	39 (8%)	68 (35%)	63 (25%)	15 (30%)	31 (31%)
	P-value	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
Lipid lowering drugs	1990	45 (4%)	46 (3%)	13 (2%)	33 (3%)	0 (0%)	1 (0.2%)
	2000	76 (14%)	84 (14%)	35 (18%)	42 (16%)	1 (2%)	13 (13%)
	P-value	<0.001	<0.001	<0.001	<0.001	0.17*	<0.001

Data are presented as mean ± standard deviation or numbers (percentages).
Percentages are calculated without missing data. For all reported variables missings occurred in <5% of all participants.
Linear or logistic regression models, adjusted for age, were used to compare the differences
* Fisher's exact test, unadjusted

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

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

IRR= incidence rate ratio, CI= confidence interval
Incidence rates are per 1000 person-years
Incidence rates and IRR are adjusted for age

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

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

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

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

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

SE= standard error, WML= white matter lesions
‡ p-values based on analyses with WML natural log transformed
Means are adjusted for age, using ANOVA

In women, the difference was significant and virtually the same across all age strata, whereas in men lacunar infarcts were less present in2005–2006 compared to 1995–1996 in the age strata 60–69 and 70–79, but more present in 2005–2006 in the highest age stratum (data not shown). In men, these differences were all nonsignificant (*p* values>0.40).

DISCUSSION

We found lower incidence rates of dementia in the 2000 subcohort than in the 1990 subcohort, albeit not significant. Evidence for a declining trend in dementia incidence was further supported by the observation that participants from the 2000 subcohort had on average less brain atrophy and less cerebral small vessel disease on MRI. Our results are in line with a recent report on trends in the incidence and prevalence of dementia in the United States, where a decline in dementia incidence was observed in Rochester between 1985 and 1994.[10] Earlier reports from Rochester reported no change in incidence between 1960 and 19745 and a possible increase of dementia among persons 85 years and older between 1965 and 1984.[8] The reported increase, however, might very well be the result of an increasing awareness of dementia and an increased case identification over the years.[8] The change in dementia incidence rates between 1985 and 1994 was a 3% decline per year, or a 30% decline in 10 years,[10] which is in line with our overall reduction of 25% in 10 years. Interestingly, in the Chicago Health and Aging Project, the effect estimate of annual change in

incidence between 1997 and 2008 was similar to the estimate found in Rochester, although not significant.[4,10] Although the reduction in dementia incidence rates in our study was also not significant at the conventional $\alpha = 0.05$ level, we think our estimates reflect a true decline in incidence rates. First, there was a risk reduction across all strata of gender and age, but for men above 80 years. Second, we also found a clear reduction in mortality rates. A person who dies is no longer at risk of developing dementia, while underlying risk factors, especially vascular factors, are associated with both dementia and mortality risk. The mortality rate, and thereby the competing risk effect,[22] was higher in the 1990 subcohort. Should mortality rates have been the same in both subcohorts, the difference in incidence rates of dementia would probably have been larger. Third, we used the same methods of diagnosing dementia for both subcohorts, and do not think that we underestimated the incidence in the 2000 subcohort. However, awareness and reporting of dementia by physicians have increased over the last decades. To the extent that this has influenced our study, it will have led to an increased case identification in the 2000 subcohort, and consequently an underestimating of the difference in incidence rates between both subcohorts. Finally, the larger brain volumes and lower presence of cerebral small vessel disease we observed comparing scans of participants without dementia made in 1995–1996 with scans made in 2005–2006 support our finding of declining dementia incidence. Larger brain volumes suggest less brain atrophy, and brain atrophy and cerebral small vessel disease are associated with a higher risk of dementia.[23] There are several possible explanations for our observation of a decreasing incidence of dementia. First, the 2000 subcohort was higher educated and higher education has been associated with a later onset of dementia.[24–26] However, the level of education may not adequately reflect the cognitive abilities of a person, when comparing 2 different birth cohorts. The older cohort probably had fewer possibilities to continue education after primary school, regardless of their intellectual abilities. A second explanation, as proposed for the decreasing incidence of stroke, is the implementation of preventive treatments and reduction in vascular risk factors at the population level.[11,12] In our study, however, apart from current smoking, vascular risk factors were more prevalent in the 2000 subcohort compared to the 1990 subcohort. This was, however, paralleled by a strong increase in use of antithrombotic and lipid-lowering drugs. Use of statins has been associated with a lower risk of dementia in our cohort[27] and others,[28,29] although the effect might be restricted to persons younger than 80,[29] and both antithrombotics and lipid-lowering drugs are preventive treatments for cerebrovascular disease.[30] Our observation of less brain atrophy and small vessel disease on the MRI scans in the 2000 subcohort supports the notion that a better treatment of vascular risk factors could explain the decrease in dementia incidence. The hypothesis that treatment of vascular factors might lower dementia risk has become more popular in recent years,[26,31] but thus far, dementia has mostly been investigated as a secondary endpoint in clinical trials that were underpowered to assess a moderate

reduction in dementia risk. However, a meta-analysis suggested a 13% risk reduction by antihypertensivetreatment,[32] which is an effect size not unlike many other effects seen in the cardiovascular field.[33] Finally, a decline in stroke incidence itself could also attribute to a decreasing incidence of dementia, because stroke is associated with a higher risk of dementia independent of other vascular risk factors.[34] Important strengths of our study are the comparison of 2 large independent subcohorts from one population-based study, the equal assessment of dementia and vascular risk factors in both subcohorts, and the virtually complete follow-up for both subcohorts. The Dutch health care system and close collaboration with the general practitioners allowed for continuous monitoring for incident dementia through medical records, even when participants did not participate in the follow-up visits. We had a limited number of dementia cases in the 2000 subcohort, where the age distribution was more skewed toward younger people. We accounted for this difference in age distribution by adjusting forage, additional adjustments for age squared, and by analyzing in different age strata. The fact that we had only 49 cases in the 2000 subcohort not only limited our statistical power, but also precluded us looking at different subtypes of dementia. As in every observational study there remain some uncertainties in interpreting the findings, due to the possibility of residual confounding or sampling effects, especially in the imaging study. Finally, despite similar postprocessing, we used different MRI scanners in 1995–1996and 2005–2006, which could have affected the comparison. We consider it unlikely, however, that this would have led to a consistent overestimation of total brain volume and at the same time a consistent underestimation of WML and lacunar infarcts in 2005–2006 compared to 1995–1996. Our study suggests that the dramatic rise in absolute numbers of people living with dementia in the coming years may be slightly less enormous than has previously been reported.

REFERENCES

1. Ferri CP, Prince M, Brayne C, et al. Global prevalence of dementia: a Delphi consensus study. Lancet 2005;366:2112–2117.
2. Jagger C, Matthews R, Lindesay J, Robinson T, Croft P, Brayne C. The effect of dementia trends and treatments on longevity and disability: a simulation model based on the MRC Cognitive Function and Ageing Study (MRCCFAS). Age Ageing 2009;38:319 –325.
3. Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. Alzheimers Dement 2007;3:186 –191.
4. Hebert LE, Bienias JL, Aggarwal NT, et al. Change in risk of Alzheimer disease over time. Neurology 2010;75:786–791.
5. Kokmen E, Chandra V, Schoenberg BS. Trends in incidence of dementing illness in Rochester, Minnesota, in three quinquennial periods, 1960–1974. Neurology 1988;38:975–980.
6. Li S, Yan F, Li G, et al. Is the dementia rate increasing in Beijing? Prevalence and incidence of dementia 10 years later in an urban elderly population. Acta Psychiatr Scand 2007;115:73–79.
7. Rorsman B, Hagnell O, Lanke J. Prevalence and incidence of senile and multi-infarct dementia in the Lundby Study: a comparison between the time periods 1947–1957 and1957–1972. Neuropsychobiology 1986;15:122–129.
8. Kokmen E, Beard CM, O'Brien PC, Offord KP, Kurland LT. Is the incidence of dementing illness changing? A 25-year time trend study in Rochester, Minnesota (1960–1984). Neurology 1993;43:1887–1892.
9. Ukraintseva S, Sloan F, Arbeev K, Yashin A. Increasing rates of dementia at time of declining mortality from stroke. Stroke 2006;37:1155–1159.
10. Rocca WA, Petersen RC, Knopman DS, et al. Trends in the incidence and prevalence of Alzheimer's disease, dementia, and cognitive impairment in the United States. Alzheimers Dement 2011;7:80 –93.
11. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. Lancet Neurol 2009;8:355–369.
12. Rothwell PM, Coull AJ, Giles MF, et al. Change in stroke incidence, mortality, case-fatality, severity, and risk factors in Oxfordshire, UK from 1981 to 2004 (Oxford VascularStudy). Lancet 2004;363:1925–1933.
13. Middleton LE, Yaffe K. Targets for the prevention of dementia. J Alzheimers Dis 2010;20:915–924.
14. Hofman A, van Duijn CM, Franco OH, et al. The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol2011;26:657– 686.
15. Ott A, Breteler MM, van Harskamp F, Stijnen T, HofmanA. Incidence and risk of dementia: The Rotterdam Study. Am J Epidemiol 1998;147:574 –580.
16. Folstein MF, Folstein SE, McHugh PR. "Mini-mentalstate": a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–198.
17. Copeland JR, Kelleher MJ, Kellett JM, et al. A semistructured clinical interview for the assessment of diagnosis and mental state in the elderly: the Geriatric Mental State Schedule: I: development and reliability. Psychol Med1976;6:439–449.
18. Roth M, Tym E, Mountjoy CQ, et al. CAMDEX: a standardized instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. Br J Psychiatry 1986;149:698 –709.
19. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders: DSM-III-R. Washington, DC: American Psychiatric Association; 1987.
20. Ikram MA, Vrooman HA, Vernooij MW, et al. Brain tissue volumes in the general elderly population: The Rotterdam Scan Study. Neurobiol Aging 2008;29:882– 890.
21. Ikram MA, van der Lugt A, Niessen WJ, et al. The RotterdamScan Study: design and update up to 2012. Eur JEpidemiol 2011;26:811– 824.
22. Seshadri S, Wolf PA, Beiser A, et al. Lifetime risk of dementia and Alzheimer's disease: the impact of mortality on risk estimates in the Framingham Study. Neurology 1997;49:1498 –1504.
23. Ikram MA, Vrooman HA, Vernooij MW, et al. Brain tissue volumes in relation to cognitive function and risk of dementia. Neurobiol Aging 2010;31:378 –386.

24. Brayne C, Ince PG, Keage HA, et al. Education, the brain and dementia: neuroprotection or compensation? Brain 2010;133:2210 –2216.

25. Caamano-Isorna F, Corral M, Montes-Martinez A, Takkouche B. Education and dementia: a meta-analytic study. Neuroepidemiology 2006;26:226 –232.

26. Langa KM, Larson EB, Karlawish JH, et al. Trends in the prevalence and mortality of cognitive impairment in the United States: is there evidence of a compression of cognitive morbidity? Alzheimers Dement 2008;4:134 –144.

27. Haag MD, Hofman A, Koudstaal PJ, Stricker BH, BretelerMM. Statins are associated with a reduced risk of Alzheimer disease regardless of lipophilicity: The Rotterdam Study. J Neurol Neurosurg Psychiatry 2009;80:13–17.

28. Cramer C, Haan MN, Galea S, Langa KM, Kalbfleisch JD. Use of statins and incidence of dementia and cognitive impairment without dementia in a cohort study. Neurology2008;71:344 –350.

29. Li G, Shofer JB, Rhew IC, et al. Age-varying association between statin use and incident Alzheimer's disease. J Am Geriatr Soc 2010;58:1311–1317.

30. Goldstein LB, Adams R, Alberts MJ, et al. Primary prevention of ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council: cosponsored by the Atherosclerotic Peripheral Vascular Disease Interdisciplinary Working Group; Cardiovascular Nursing Council; Clinical Cardiology Council; Nutrition, Physical Activity, and Metabolism Council; and the Quality of Care and Outcomes Research Interdisciplinary Working Group. Circulation 2006;113:e873– e923.

31. Viswanathan A, Rocca WA, Tzourio C. Vascular risk factorsand dementia: how to move forward? Neurology2009;72:368 –374.

32. Peters R, Beckett N, Forette F, et al. Incident dementiaand blood pressure lowering in the Hypertension in theVery Elderly Trial cognitive function assessment (HYVET-COG): a double-blind, placebo controlled trial. Lancet Neurol 2008;7:683– 689.

33. Sacco RL, Diener HC, Yusuf S, et al. Aspirin andextended-release dipyridamole versus clopidogrel for recurrentstroke. N Engl J Med 2008;359:1238 –1251.

34. Leys D, Henon H, Mackowiak-Cordoliani MA, Pasquier F. Poststroke dementia. Lancet Neurol 2005;4:752–759.

Chapter 4.2

The relation of uric acid to brain atrophy and cognition:

The Rotterdam Scan Study

Benjamin F. J. Verhaaren, Meike W. Vernooij, Abbas Dehghan, Henri A. Vrooman, Renske de Boer, Albert Hofman, Jacqueline C. M. Witteman, Wiro J. Niessen, Monique M. B. Breteler, Aad van der Lugt, M. Arfan Ikram.

Neuroepidemiology. 2013;41(1):29-34.

ABSTRACT

Background: Uric acid has been associated with focal vascular brain disease. However, it is unknown whether uric acid also relates to global brain changes such as brain atrophy. We therefore studied relation of uric acid with brain atrophy and whether this is accompanied by worse cognitive function.

Methods: In 814 persons of the population-based Rotterdam Study (mean age 62.0 years), we studied the relation of uric acid levels with brain tissue atrophy and cognition, using linear regression models, adjusted for age, sex and putative confounders. Brain atrophy was assessed using automated processing of MRI. Cognition was assessed using a validated neuropsychological test-battery and we computed compound scores of cognitive domains.

Results: Higher uric acid levels were associated with white matter atrophy (difference in Z-score of white matter volume per standard deviation increase in uric acid: -0.07 (95% confidence interval: -0.12; -0.01)), but not with grey matter atrophy. This was particularly marked when comparing hyperuricemic to normo-uricemic persons (Z-score difference: -0.27 (-0.43; -0.11)). Worse cognition was primarily found in persons with hyperuricemia (-0.28 (-0.48; -0.08)).

Conclusions: Hyperuricemia is related to white matter atrophy and worse cognition.

INTRODUCTION

Vascular risk factors play an important role in the etiology of dementia, including Alzheimer's disease.[1,2] The underlying mechanism is thought to involve subclinical vascular brain disease, which can be visualized using magnetic resonance imaging (MRI).[3] Vascular brain disease may be present years before clinical onset of dementia and can lead to subtle deficits in cognition.[3] In recent years, uric acid has been reappraised as a vascular risk factor and considered to have an effect independently from established risk factors.[4] Several studies have shown higher uric acid levels to be a risk factor for not only cardiac disease, but also cerebrovascular disease, including clinical stroke.[4-6] With respect to subclinical vascular disease, uric acid has been associated with focal MRI markers, such as white matter lesions and lacunar infarcts.[7-9] However, recent studies show that MRI markers of vascular brain disease also include global brain changes such as brain atrophy, in particular white matter atrophy.[10] In turn, brain atrophy has also been associated with cognition.[3,10] Studies investigating the association of uric acid with brain atrophy are lacking.

We therefore studied the relationship between uric acid and brain atrophy and whether this is accompanied by worse cognitive function in a large non-demented aging population.

MATERIAL AND METHODS

Study population

The study is based on participants from the Rotterdam Study, a population-based cohort study in the Netherlands aimed at investigating determinants of various chronic diseases among elderly persons.[11] The original study population (n = 7,983) consisted of the general population aged 55 years and older within the Ommoord area, a suburb of Rotterdam. In 2000, the cohort was expanded with 3,011 persons (≥ 55 years) who were living in the study area and had not been included before.[11] From August 2005 to May 2006, we randomly selected 1,073 members of this cohort expansion for participation in the Rotterdam Scan Study[12], a prospective brain MRI study that is embedded within the Rotterdam Study. The institutional review board (Erasmus MC, Rotterdam, the Netherlands) approved the study. We excluded individuals with dementia (n=4) or those who had MRI contraindications (n = 94). A total of 975 persons were eligible, of whom 907 participated and gave written informed consent. Because of physical inabilities, imaging could not be performed in 12 individuals. From the remaining participants 35 had to be excluded because of imaging artefacts, leaving a total of 860 persons with complete MRI measurements. From these individuals, 19 persons with cortical infarcts on MRI were excluded. Finally, from these persons, 814 had uric acid measurements at baseline and were available for the current analyses.

Uric acid

Non-fasting blood was collected and within 30 minutes the blood was centrifuged for 10 min at 3000 r.p.m. Subsequently, the serum was stored at −20°C for 1 week until uric acid activity (kU/L) was determined with a Kone Diagnostica reagent kit and a Kone autoanalyzer.[13] To check calibration, after every 10 samples, three control samples were included; if the average values of the control samples of each run (100 samples) were not within 2.5% of the true value, the run was repeated. Day-by-day variation had to be within 5%.

Magnetic resonance imaging

Brain magnetic resonance imaging (MRI) was performed on a 1.5 Tesla scanner (GE Healthcare) with an eight channel head coil and included T1 weighted, proton-density weighted and fluid-attenuated inversion recovery (FLAIR) sequences.[12] Post-processing steps have been described elsewhere, and include a conventional k-nearest-neighbour brain tissue classifier extended with white matter lesion segmentation.[14,15] Using this classifier we obtained quantitative measures of brain volume, white matter volume, grey matter volume, white matter lesion volume and intracranial volume.

Presence of infarcts on MRI was assessed on structural MRI images. Lacunar infarcts were defined as focal lesions 3 mm or more and less than 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 sequence.[16] Infarcts showing involvement of cortical gray matter were classified as cortical infarcts.

Assessment of cognitive function

Cognitive function was assessed with the following neuropsychological

test battery: a 15-word verbal learning test (15-WLT, based on the Rey recall of words),[17] the Stroop test,[18] the Letter-Digit Substitution Task (LDST),[19] and a word fluency test (animal categories).[20] We generated Z-scores (individual test score minus mean test score divided by the standard deviation) for each cognitive test. To obtain more robust measures, we constructed compound scores for memory, executive function, information processing speed, and global cognition. The Z-scores for the Stroop tasks were inverted for use in these compound scores, as higher scores on the Stroop task indicate a worse performance while higher scores on all other tests indicate a better cognitive function. The compound score for memory was the average of the Z-scores for the immediate and delayed recall of the 15-WLT. Executive function was constructed by averaging the Z-scores for the Stroop interference subtask, the LDST (number of correct digits in 1 minute), and the word fluency test (number of animals in 1 minute). Information processing speed was the average of the Z-scores for the Stroop reading and Stroop color naming test and the LDST. For global cognitive function,

we used the average of the Z-scores of the Stroop task (average of all 3 subtasks), the LDST, the word fluency test, and the immediate and delayed recall of the 15-WLT.

Potential confounders

Estimated glomerular filtration rate, systolic and diastolic blood pressure, body mass index, total cholesterol, HDL-cholesterol, diabetes mellitus, diuretic use, alcohol consumption (units per week) and smoking (never, previous, current) were determined as previously described.[5,21] Use of cytostatic drugs and medication for gout were assessed during a home interview. The attained level of education was assessed by interview according to the standard classification of education.[22] In our analysis, we combined the 4 highest levels into 1 category, thus, obtaining 4 levels: (1) primary education; (2) low-level vocational training; (3) medium level secondary education; and (4) medium-level vocational training to university level.

Statistical analyses

We used linear regression models to investigate the relationship of uric acid levels with brain tissue volumes (total brain, gray matter and white matter), separate cognitive tests, as well as compound scores of cognition (global cognition, memory, executive function and information processing speed). These compound scores have been published on previously [22-24] and were corroborated by a principal component analysis. For interpretation and comparability of the effect estimates, Z-scores were computed for continuous outcomes. We computed sex-specific Z-scores of uric acid levels, because the distribution of clinically normal levels of uric acid differs per sex.[25] All models were adjusted for age and sex, and additionally for glomerular filtration rate, systolic and diastolic blood pressure, body mass index, total cholesterol, HDL-cholesterol, diabetes mellitus, diuretic use, alcohol consumption and smoking. Models with brain tissue volumes as outcome were adjusted for intracranial volume. Models with cognitive function as outcome were additionally adjusted for level of education. To evaluate a possible role of brain atrophy as mediator of the relationship of uric acid with cognitive function, we additionally adjusted for markers of vascular brain disease (brain tissue volumes, white matter lesions and lacunar infarcts).

We further explored the associations over different categories of uric acid levels to evaluate whether any associations found were driven by hyperuricemia. For this, we divided the population in normo-uricemic and hyperuricemic persons (men: uric acid levels > 420 µmol/L, women: uric acid levels > 360 µmol/L)[26] and, further, constructed quartiles of uric acid levels among the normo-uricemic population.

Finally, we repeated analyses excluding persons receiving medical therapy for gout (N=3), persons receiving cytostatic treatment (N=3), or persons with hypo-uricemia (uric acid levels < 120 µmol/L)[27] (N=1).

RESULTS

Population characteristics are represented in Table 1. Mean age was 62.0 (standard deviation: 5.4) years and 50.9% were women. Brain MRI characteristics and cognitive test results can be found in online supplementary table S1 and S2 (http://www.karger.com/Journal/Home/224263), respectively.

Associations of uric acid levels with brain tissue volumes and cognition are shown in Table 2. Higher uric acid levels were associated with smaller brain volume, in particular with smaller white matter volume (difference in Z-score per standard deviation increase of uric acid level: -0.07 (95%-confidence interval: -0.12; -0.01), independent of putative confounders). No significant associations were seen with smaller grey matter volume. Higher uric acid levels were associated with worse global cognitive function, primarily driven by information processing speed (-0.09 (-0.16; -0.03)) and to a lesser extent executive function. Online supplementary table S3 shows the relationship of uric acid with the separate cognitive tests. Figure 1 shows categories of uric acid levels in relation to white matter volume and information processing speed, after stratification by hyperuricemia (n = 87) versus normo-uricemia (n = 727). This revealed that hyperuricemia was strongly related to smaller white matter volume (difference in Z-score of white matter volume between persons with hyperuricemia versus normo-uricemia: -0.27 (-0.43; -0.11)). Conversely, among individuals with normo-uricemia there was no relationship between uric acid levels and white matter volume (difference in Z-score per standard deviation increase of uric acid level: -0.01 (-0.07; 0.04)).

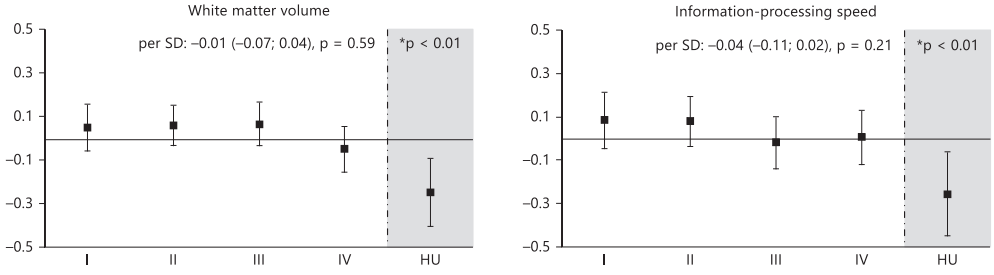

Figure 1. Y-axis scale indicates mean Z-scores of white matter volume and information processing speed, respectively.

Values are adjusted for age, sex and glomerular Filtration rate, systolic and diastolic blood pressure, body mass index, total cholesterol, HDL-cholesterol, diabetes mellitus, diuretic use, alcohol consumption and smoking. Mean Z-scores of white matter volume are additionally adjusted for intracranial volume. Mean Z-scores of information processing speed are additionally adjusted for level of education. X-axis scale indicates categories of uric acid level, in which category I till IV represent quartiles of normal uric acid levels and category HU represents hyperuricemia. The ranges of uric acid in µmol/L per category were as follows in women: I: 110-230, II: 240-270, III: 280-300, IV: 310-360, HU > 360, and in men: I: 120-280, II: 290-320, III: 330-360, IV: 370-420, HU > 420. *p-value for hyperuricemia versus normo-uricemia. Abbreviations: SD = standard deviation increase in uric acid levels, HU = hyperuricemia.

Table 1. Population characteristics

	n = 814
Age, yr	62.0 (5.4)
Female sex	50.9%
Uric acid, µmol/L	
Women	281 (63)
Men	342 (68)
Glomerular filtration rate, mL/min	83 (16)
Systolic blood pressure, mmHg	139 (20)
Diastolic blood pressure, mmHg	78 (10)
Total cholesterol, mmol/L	5.8 (0.9)
High Density Lipoprotein, mmol/L	1.4 (0.4)
Body mass index	26.8 (3.5)
Diabetes mellitus*	8.4%
Alcohol intake, units/week†	6 [14]
Current smoker	17.6%
Diuretic use	6.9%
Primary education only	3.5%

Values are means (standard deviation) or percentages. *defined as non-fasting glucose >11.1 mmol/L or receiving glucose lowering drugs. †Median [interquartile-range]

Table 2. Associations between uric acid levels and brain tissue volumes and cognition

	Model I Effect estimate (95% CI)		Model II Effect estimate (95% CI)	
Total brain	-0.04	(-0.06; -0.01)	-0.03	(-0.05; 0.00)
Gray matter	0.03	(-0.01; 0.07)	0.03	(-0.01; 0.08)
White matter	-0.08	(-0.13; -0.04)	-0.07	(-0.12; -0.01)
Global cognition	-0.06	(-0.11; -0.02)	-0.08	(-0.14; -0.03)
Memory	-0.05	(-0.12; 0.01)	-0.07	(-0.15; 0.01)
Executive function	-0.06	(-0.12; -0.01)	-0.09	(-0.15; -0.03)
Information processing speed	-0.08	(-0.13; -0.02)	-0.09	(-0.16; -0.03)

Values are differences in Z-score per standard deviation increase in uric acid level with corresponding 95% confidence intervals (CI).
Model I: adjustments for age and sex.
Model II: as in I, additionally adjusted for other potential confounders (glomerular filtration rate, systolic and diastolic blood pressure, body mass index, total cholesterol, HDL-cholesterol, diabetes mellitus, diuretic use, alcohol consumption and smoking).
Brain tissue volumes are additionally adjusted for intracranial volume. Cognitive Z-scores are additionally adjusted for level of education.

Similarly, the relationship between uric acid levels and information processing speed was not statistically significant in the normo-uricemic population (-0.04 (-0.11; 0.02)), but showed significance when hyperuricemic persons were compared to normo-uricemic persons (-0.28 (-0.48; -0.08)). This association attenuated but remained significant after adjustment for

global and focal markers of vascular disease (white matter volume, white matter lesions and lacunar infarcts): -0.24 (-0.44; -0.04). Similar associations were found the analyses with respect to executive function. In supplementary figure S1 and S2 the categories of uric acid levels in relation to total brain volume, gray matter volume, global cognition, memory and executive function are shown. Paradoxically here we found that hyperuricemia was related to relatively more gray matter volume.
Exclusion of persons receiving gout-medication, cytostatics or having hypouricemia did not influence any of the results.

DISCUSSION

In this study we found uric acid to be associated with white matter atrophy and worse cognition. More specifically, we found that the associations with white matter atrophy and worse cognition were predominantly evident when comparing hyperuricemic persons to normo-uricemic persons.
Strengths of our study are its population based setting, large sample size and quantitative assessment of atrophy of different brain tissues. Furthermore we assessed cognitive function in various domains using standardized neuropsychological tests. A potential limitation of the study is the absence of longitudinal brain imaging, making the interpretation of cause and effect difficult. Another consideration is that we measured serum uric acid only once, ignoring possible intra-individual fluctuations in serum uric acid levels. This may have caused an underestimation of the estimates.
Our findings that hyperuricemia relates to white matter atrophy, a marker of global vascular brain disease, is in line with studies that found uric acid related to focal vascular brain disease.[5,7-9] Those studies found high levels of uric acid related to clinical stroke, cerebral white matter lesions and silent brain infarcts.[5,7-9] To our knowledge no studies investigated the relation between uric acid and brain atrophy.
Surprisingly the association between hyperuricemia and white matter atrophy was accompanied by a relative increase in gray matter. However, we found that this was irrespective of the association with uric acid and would not survive a multiple testing correction. We regard this as an artefact of imaging and segmentation processing. There are two potential explanations for this. One is that white matter atrophy may result on imaging in signal changes in white matter, approaching the signal intensity of gray matter. This phenomenon has been reported to occur with aging[28] and may cause the border between the gray and white matter segmentation to shift towards the white matter. A second explanation is that with atrophy the gyri move away from the overlying dura, which may lead to a more defined outer border and better/more gray matter segmentation.

Our finding that uric acid relates to worse cognition is supported by most studies.[7,24,29,30] Moreover, of all cognitive domains investigated, we found strongest associations for information processing speed and executive function. This corresponds to the literature, which shows these domains to be particularly affected by cardiovascular risk factors.[31] Nevertheless, we also note that other studies have found uric acid to be related to better cognition or even to a decreased risk of dementia.[24,32] Also, beneficial effects have been found for Parkinson's disease and Parkinson's disease related cognitive decline.[33] The biologic explanation for these associations is that uric acid is also a strong antioxidant.[4] It remains unclear what the net effect of these opposing effects is on brain function. A possibility may be that the beneficial effect of uric acid is present within the normal range, whilst the detrimental effect is more pronounced in hyperuricemic states. Still, more research is needed to elucidate this issue.

Whether vascular brain disease could explain the association between uric acid and cognition has only been studied once. In this study of 180 people, the authors found a relation between uric acid and worse cognition that disappeared after taking into account focal vascular brain disease (white matter lesions).[7] In our study the relation between uric acid and cognition attenuated but still remained significant after taking into account white matter atrophy and focal markers of vascular brain disease. This suggests that the relation of uric acid with cognition is not solely via atrophy or focal vascular brain disease. Therefore perhaps subtle brain abnormalities explain more of the relationship between uric acid and cognition, such as microinfarcts that are not visible on structural MRI-scans.[34] Possibly the smaller sample size of the previous study - and therefore less statistical power - explains why that study did not find an association with cognition independent from vascular brain disease.

In conclusion, we found that hyperuricemia relates to white matter atrophy and worse cognition. Further studies are needed to determine causality and to further explore the exact role of uric acid in neurodegenerative disease. This could then lead to potential preventive or therapeutic strategies targeting uric acid levels.

REFERENCES

1. Schneider JA, Bennett DA. Where vascular meets neurodegenerative disease. *Stroke.* Oct 2010;41(10 Suppl):S144-146.
2. Knopman DS, Roberts R. Vascular risk factors: imaging and neuropathologic correlates. *J Alzheimers Dis.* 2010;20(3):699-709.
3. Ikram MA, Vrooman HA, Vernooij MW, et al. Brain tissue volumes in relation to cognitive function and risk of dementia. *Neurobiol Aging.* Mar 2010;31(3):378-386.
4. Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. *N Engl J Med.* Oct 23 2008;359(17):1811-1821.
5. Bos MJ, Koudstaal PJ, Hofman A, Witteman JC, Breteler MM. Uric acid is a risk factor for myocardial infarction and stroke: the Rotterdam study. *Stroke.* Jun 2006;37(6):1503-1507.
6. Hozawa A, Folsom AR, Ibrahim H, Javier Nieto F, Rosamond WD, Shahar E. Serum uric acid and risk of ischemic stroke: the ARIC Study. *Atherosclerosis.* Aug 2006;187(2):401-407.
7. Vannorsdall TD, Jinnah HA, Gordon B, Kraut M, Schretlen DJ. Cerebral ischemia mediates the effect of serum uric acid on cognitive function. *Stroke.* Dec 2008;39(12):3418-3420.
8. Schretlen DJ, Inscore AB, Vannorsdall TD, et al. Serum uric acid and brain ischemia in normal elderly adults. *Neurology.* Oct 2 2007;69(14):1418-1423.
9. Heo SH, Lee SH. High levels of serum uric acid are associated with silent brain infarction. *J Neurol Sci.* Oct 15 2010;297(1-2):6-10.
10. Debette S, Seshadri S, Beiser A, et al. Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. *Neurology.* Aug 2 2011;77(5):461-468.
11. Hofman A, van Duijn CM, Franco OH, et al. The Rotterdam Study: 2012 objectives and design update. *Eur J Epidemiol.* Aug 30 2011.
12. Ikram MA, van der Lugt A, Niessen WJ, et al. The Rotterdam Scan Study: design and update up to 2012. *Eur J Epidemiol.* Oct 16 2011.
13. Trivedi RC, Rebar L, Desai K, Stong LJ. New ultraviolet (340 nm) method for assay of uric acid in serum or plasma. *Clin Chem.* Apr 1978;24(4):562-566.
14. Vrooman HA, Cocosco CA, van der Lijn F, et al. Multi-spectral brain tissue segmentation using automatically trained k-Nearest-Neighbor classification. *NeuroImage.* Aug 1 2007;37(1):71-81.
15. de Boer R, Vrooman HA, van der Lijn F, et al. White matter lesion extension to automatic brain tissue segmentation on MRI. *Neuroimage.* May 1 2009;45(4):1151-1161.
16. Kruit MC, Launer LJ, Ferrari MD, van Buchem MA. Infarcts in the posterior circulation territory in migraine. The population-based MRI CAMERA study. *Brain.* Sep 2005;128(Pt 9):2068-2077.
17. Bleecker ML, Bolla-Wilson K, Agnew J, Meyers DA. Age-related sex differences in verbal memory. *J Clin Psychol.* May 1988;44(3):403-411.
18. Golden CJ. Identification of brain disorders by the Stroop Color and Word Test. *J Clin Psychol.* Jul 1976;32(3):654-658.
19. Lezak MD HD, Loring DW. *Neuropsychological assesment.* New York, NY: Oxford University Press; 2004.
20. Welsh KA, Butters N, Mohs RC, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part V. A normative study of the neuropsychological battery. *Neurology.* Apr 1994;44(4):609-614.
21. Ikram MA, Vernooij MW, Hofman A, Niessen WJ, van der Lugt A, Breteler MM. Kidney function is related to cerebral small vessel disease. *Stroke.* Jan 2008;39(1):55-61.
22. Verhaaren BF, Vernooij MW, Koudstaal PJ, et al. Alzheimer's Disease Genes and Cognition in the Nondemented General Population. *Biol Psychiatry.* May 14 2012.
23. Euser SM, Schram MT, Hofman A, Westendorp RG, Breteler MM. Measuring cognitive function with age: the influence of selection by health and survival. *Epidemiology.* May 2008;19(3):440-447.
24. Euser SM, Hofman A, Westendorp RG, Breteler MM. Serum uric acid and cognitive function and dementia. *Brain.* Feb 2009;132(Pt 2):377-382.
25. Hall AP, Barry PE, Dawber TR, McNamara PM. Epidemiology of gout and hyperuricemia. A long-term population study. *Am J Med.* Jan 1967;42(1):27-37.

26. Chang HY, Tung CW, Lee PH, et al. Hyperuricemia as an independent risk factor of chronic kidney disease in middle-aged and elderly population. *Am J Med Sci.* Jun 2010;339(6):509-515.

27. Ogino K, Hisatome I, Saitoh M, et al. Clinical significance of hypouricemia in hospitalized patients. *J Med.* 1991;22(2):76-82.

28. Salat DH, Lee SY, van der Kouwe AJ, Greve DN, Fischl B, Rosas HD. Age-associated alterations in cortical gray and white matter signal intensity and gray to white matter contrast. *Neuroimage.* Oct 15 2009;48(1):21-28.

29. Schretlen DJ, Inscore AB, Jinnah HA, Rao V, Gordon B, Pearlson GD. Serum uric acid and cognitive function in community-dwelling older adults. *Neuropsychology.* Jan 2007;21(1):136-140.

30. Afsar B, Elsurer R, Covic A, Johnson RJ, Kanbay M. Relationship between Uric Acid and Subtle Cognitive Dysfunction in Chronic Kidney Disease. *Am J Nephrol.* Jun 10 2011;34(1):49-54.

31. Scuteri A, Nilsson PM, Tzourio C, Redon J, Laurent S. Microvascular brain damage with aging and hypertension: pathophysiological consideration and clinical implications. *J Hypertens.* Aug 2011;29(8):1469-1477.

32. Alvarez-Lario B, Macarron-Vicente J. Uric acid and evolution. *Rheumatology (Oxford).* Nov 2010;49(11):2010-2015.

33. Wirdefeldt K, Adami HO, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. *Eur J Epidemiol.* Jun 2011;26 Suppl 1:S1-58.

34. Brundel M, de Bresser J, van Dillen JJ, Kappelle LJ, Biessels GJ. Cerebral microinfarcts: a systematic review of neuropathological studies. *J Cereb Blood Flow Metab.* Mar 2012;32(3):425-436.

Chapter 4.3

High blood pressure and cerebral white matter lesion progression in the general population

Benjamin F.J. Verhaaren, Meike W. Vernooij, Renske de Boer, Albert Hofman,
Wiro J. Niessen, Aad van der Lugt, M. Arfan Ikram.

Hypertension. 2013 Jun;61(6):1354-9.

ABSTRACT

High blood pressure is considered an important risk factor for cerebral white matter lesions in aging. In a longitudinal population-based study of 665 non-demented persons, we investigated the longitudinal relationship of systolic blood pressure, diastolic blood pressure and pulse pressure with annual progression of white matter lesions. Means of blood pressure were calculated over a 5-year period prior to longitudinal magnetic resonance imaging scanning. White matter lesion progression was subsequently measured on two scans 3.5 years apart. We performed analyses with linear regression models and evaluated adjustments for age, sex, cardiovascular risk factors and baseline white matter lesion volume. In addition, we evaluated whether treatment of hypertension is related to less white matter lesion progression. Both systolic and diastolic blood pressure were significantly associated with annual white matter lesion progression (regression coefficient (95%-confidence interval): 0.08 (0.03; 0.14) mL/year and 0.09 (0.03; 0.15) mL/year per standard deviation increase in systolic and diastolic blood pressure, respectively. Pulse pressure was also significantly associated with white matter lesion progression, but not independent from hypertension. After adjustment for baseline white matter lesion volume, only systolic blood pressure remained significantly associated: 0.05 (0.00; 0.09) mL/year per standard deviation increase. People with uncontrolled untreated hypertension had significantly more white matter lesion progression than people with uncontrolled treated hypertension (difference (95%-confidence interval): 0.12 (0.00; 0.23) mL/year. The present study further establishes high blood pressure to precede white matter lesions and implies hypertension treatment could reduce white matter lesion progression in the general population.

INTRODUCTION

Cerebral white matter lesions (WMLs) are highly prevalent in the elderly population and increase the risk of dementia and stroke.[1] Although believed to be vascular in origin, the exact etiology of WMLs is still unknown. Based on pathological and epidemiological studies, blood pressure is considered to be one of the most important factors by damaging the cerebral small vessels.[2-3] Since blood pressure is modifiable, blood pressure control seems an important candidate for the prevention of WML progression.

The earliest studies demonstrating an association between high blood pressure and WMLs were cross-sectional by design, which limits causal inferences.[4-13] More recently, studies have employed longitudinal designs and found similar results.[14-23] Yet, since WML progression is strongly influenced by the WML load at baseline,[15] it is unknown to what extent associations of blood pressure with WML progression are affected by the baseline WML load. Moreover, to provide stronger evidence for a temporal relationship, blood pressure should preferably be measured *prior* to the window in which WML progression is determined, instead of *during* this window. In addition, the use of different magnetic resonance imaging (MRI) scanners or scanning protocols when measuring WML progression can possibly lead to systematic biases. Previous studies have addressed one or two of these issues, but none of them addressed all.

It is also unknown whether the associations between blood pressure and WML progression are present for systolic, diastolic as well as pulse pressure. Moreover, the influence of medication use and control of hypertension on WML progression remains unclear. We hypothesized that blood pressure would relate to WML progression even when taking baseline WML load into account, and that medication use and adequate control of hypertension would reduce this progression.

We tested this hypothesis in a population-based longitudinal MRI study in which we measured systolic, diastolic and pulse pressure prior to MRI scanning; evaluated the influence of the WML load at baseline; and used exactly the same scanners and scanning protocol at baseline and follow-up.

METHODS

Study population and design

The study is based on participants from the Rotterdam Study, a population-based cohort study in the Netherlands that investigates determinants of various chronic diseases among elderly people.[24] The original study population consisted of the general population aged 55 years and older within the Ommoord area, a suburb of Rotterdam. In 2000, the cohort was

expanded with 3,011 people (≥ 55 years) who were living in the study area and had not been included before.[24]

For this report we used data from a random subset of the latter cohort expansion, which underwent three visits: visit 1 (in the year 2000), visit 2 (2005-2006) and visit 3 (2008-2010). At visit 1 and 2, blood pressure and cardiovascular determinants were assessed. At visit 2 and 3, MRI-scanning was performed.[25] Figure 1 displays the design employed and the timings of the various longitudinal measurements. No blood pressure measurements were available at visit 3.

At visit 2, 1,073 people were randomly selected for this study from the full cohort expansion (n=3,011), because MRI-scanning was implemented only from visit 2 onwards and could not be performed in the full cohort. Hereto, we used a simple random sampling procedure. After excluding people with previous clinical stroke (n = 35), dementia (n = 4) or those who had MRI contraindications (n = 94), a total of 944 people was eligible. From these, 877 participated and gave written informed consent. Because of physical inabilities, imaging could not be performed in 12 persons, leaving 865 people that underwent MRI scanning. From these, 731 people had a second MRI at visit 3, and out of these 699 people had good quality MRI-data of both scans. After excluding 19 people with MRI-defined cortical infarcts, which hampered the assessment of WMLs, and 15 people with missing information on blood pressure measures, 665 people remained for the current analysis. All measurements were performed at the Rotterdam Study research center, which is a single site.

The institutional review board (Erasmus MC, Rotterdam, the Netherlands) approved the study.

Blood pressure, hypertension and antihypertensive medication

At visit 1 and visit 2 (5.3 years later; see Figure 1), systolic and diastolic blood pressure were measured twice on the right arm with a random-zero sphygmomanometer by a trained research physician after the participant had been sitting quietly for at least 5 min. A standard cuff or, if applicable, a large cuff was used. Pulse pressure was defined as systolic blood pressure minus diastolic blood pressure. To gain robust measures of blood pressure, we computed 5-year means of blood pressure measures ((mean of blood pressure measure at visit 1 summed with the mean of blood pressure measure at visit 2, and divided by 2). Hypertension was defined as systolic blood pressure ≥ 140 or diastolic blood pressure ≥ 90 mmHg or receiving antihypertensive treatment, at either of the two visits. Antihypertensive medication was assessed during a home interview. At the research center a physician ascertained the indication for which the medication had been prescribed.

Magnetic resonance imaging and WML progression

Brain magnetic resonance imaging (MRI) was performed at visit 2 and visit 3 (3.5 years apart; see Figure 1). At both visits we used the same MRI-scanner and imaging protocol, and

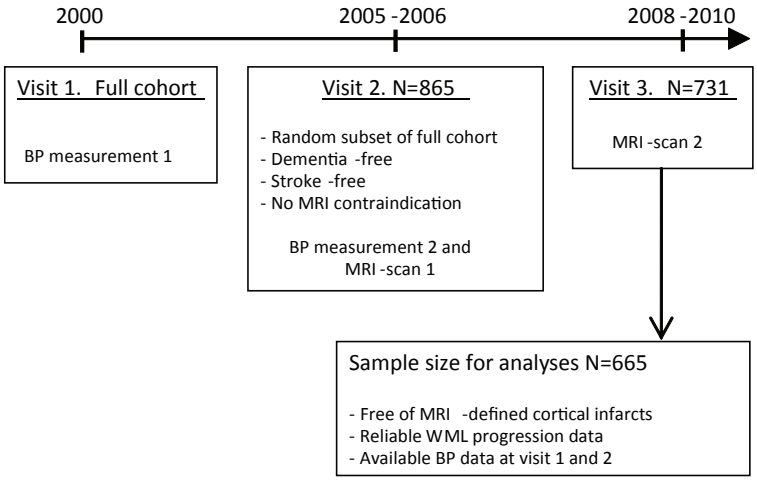

Figure 1. Schematic overview of the study design
Abbreviations: BP = blood pressure, MRI = magnetic resonance imaging, WML = white matter lesion.

applied exactly the same image post-processing steps and segmentation method. We used a 1.5 Tesla scanner (GE Healthcare) with an eight channel head coil and included T1 weighted, proton-density weighted and fluid-attenuated inversion recovery (FLAIR) sequences.[25] Post-processing steps have been described elsewhere, and include a conventional k-nearest-neighbour brain tissue classifier extended with WML segmentation.[26-27] Using this classifier we obtained quantitative measures of WML volume and intracranial volume (in mL). WML progression was assessed by subtracting the WML volume in mL at the first measurement from the WML volume in mL at the second measurement. This number was subsequently divided by the time between scans in years to obtain the annual WML progression in mL/year. Infarcts were classified as described previously.[28]

Cardiovascular risk factors

Body mass index, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides (only measured at visit 1), diabetes mellitus, alcohol consumption (only measured at visit 2) and smoking were determined by interview and laboratory and physical examination at visit 1 and 2. Body mass index was calculated by dividing weight (kg) by the square of height (m^2). The waist/hip ratio was defined as the ratio between the waist circumference (cm) by the hip circumference (cm). We considered diabetes present when a person was taking oral antidiabetics or insulin, or if fasting plasma glucose was ≥ 7 mmol/L (≥ 126 mg/dL). A physician assessed participants' smoking habits, and smoking status was further classified as current, former, or never. Alcohol intake was quantified as units per week. Cardiovascular disease

(coronary heart disease, heart failure or atrial fibrillation) was assessed during interview and verified by reviewing medical records; and through automated linkage of the study database with files from general practioners.[29] For the analyses, we computed means for the continous covariates at visit 1 and 2 and determined the ever presence of a condition between visit 1 and 2 for dichotomous variables. *ApoE*-ε4 genotyping was performed as previously described. [30]

Statistical analyses

We investigated how systolic blood pressure, diastolic blood pressure and pulse pressure were related to WML volume cross-sectionally and annual WML progression longitudinally, using linear regression models, adjusted for age, sex and intracranial volume in model I, and additionally for cardiovascular risk factors (antihypertensive medication, total cholesterol, HDL-cholesterol, triglycerides, body mass index, alcohol consumption, smoking and diabetes) in model II. For the analyses regarding WML progression, we also applied a model III with adjustments for baseline WML volume, to assess whether possible associations were explained by WML accumulated prior to the first scan.

Additionally the following exploratory analyses were conducted. We evaluated additional adjustments for cardiovascular disease and *ApoE*-ε4 carriership, and tested for interaction of *ApoE*-ε4 carriership with systolic blood pressure, diastolic blood pressure or pulse pressure. Also, we adjusted pulse pressure for the presence of hypertension to see whether pulse pressure had additional value beyond hypertension. In addition, we evaluated the addition of quadratic terms of systolic blood pressure, diastolic blood pressure and pulse pressure to explore whether J-shaped relationships were present. We repeated all analyses with waist/hip ratio as covariate instead of body mass index.

Finally, we evaluated the relation between hypertension treatment and WML progression by constructing four mutually exclusive groups of people: normotensives, controlled treated hypertensives, uncontrolled treated hypertensives and uncontrolled untreated hypertensives. These four groups were defined as follows based on the mean blood pressure of visit 1 and visit 2, and the use of antihypertensive medication during this 5-year period: 1) normotensives: study participants with normal mean blood pressure and receiving no antihypertensive treatment during this period, 2) controlled treated hypertensives: study participants with normal mean blood pressure and receiving antihypertensive treatment during this period, 3) uncontrolled treated hypertensives: study participants with hypertensive mean blood pressure, and receiving antihypertensive treatment during this period, and 4) uncontrolled untreated hypertensives: study participants with hypertensive mean blood pressure and not receiving antihypertensive treatment during this period. We compared WML progression of the groups using linear regression models, adjusted for age, sex, intracranial volume and baseline WML volume. In addition, we tested for a trend in increasing WML progression across groups.

RESULTS

Characteristics of the study population are represented in Table 1. The mean (standard deviation) age of the population was 61.6 (5.0) years at visit 1, 66.9 (5.0) years at visit 2, and 70.4 (5.0) years at visit 3. The age-range was 55-82 years at visit 1, 60-87 years at visit 2 and 64-91 years at visit 3. In online Supplementary Table S1 (http://hyper.ahajournals.org/content/61/6/1354/suppl/DC1), the associations between blood pressure measures and cross-sectionally assessed WML volume can be found.

Table 1. Characteristics of the study population

Variable	Visit 1	Visit 2	Visit 3	P-value for difference
Age, yr	61.6 (5.0)	66.9 (5.0)	70.4 (5.0)	NA
Female sex	52%	52%	52%	NA
Systolic blood pressure, mmHg	138 (19)	143 (18)	-	p < 0.01
Diastolic blood pressure, mmHg	78 (10)	81 (10)	-	p < 0.01
Pulse pressure, mmHg	60 (14)	62 (15)	-	p < 0.01
Anithypertensive medication	22%	34%	-	p < 0.01
Total cholesterol, mmol/L	5.84 (0.95)	5.73 (0.93)	-	p < 0.01
High Density Lipoprotein, mmol/L	1.39 (0.37)	1.44 (0.38)	-	p < 0.01
Triglycerides, mmol/L*	1.33 [0.80]	-	-	NA
Body mass index	26.8 (3.5)	27.5 (3.6)	-	p < 0.01
Alcohol, units/week*	-	6 [14]	-	NA
Current smoker	16%	11%	-	p < 0.01
Diabetes mellitus†	8%	9%	-	p < 0.01
Cardiovascular disease‡	6 %	7%	-	p = 0.03

Values are means (standard deviation) or percentages. *Median [interquartile-range] †defined as non-fasting glucose >11.1 mmol/L or receiving glucose lowering drugs. ‡defined as presence of coronary heart disease, heart failure or atrial fibrillation. NA = not applicable.

Table 2. Annual WML progression per standard deviation increase in blood pressure measure

Variable	Model I	Model II	Model III
Systolic blood pressure	0.07 (0.02; 0.12)	0.08 (0.03; 0.14)	0.05 (0.00; 0.09)
Diastolic blood pressure	0.07 (0.02; 0.12)	0.09 (0.03; 0.15)	0.02 (-0.02; 0.07)
Pulse pressure	0.05 (0.00; 0.10)	0.06 (0.00; 0.11)	0.04 (0.00; 0.08)

Values are white matter lesion progression in mL/year (95%-confidence interval) per standard deviation increase of blood pressure (mean of the measures at visit 1 and 2), derived from linear regression models:
Model I: adjustments for age, sex and intracranial volume.
Model II: adjustments for age, sex, intracranial volume, antihypertensive medication, total cholesterol, HDL-cholesterol, triglycerides, body mass index, alcohol consumption, smoking and diabetes.
Model III: as model II, but additional adjustment for WML volume on scan 1. Abbreviations: WML = white matter lesion, HDL = High-density lipoprotein.

Table 2 shows the relation of systolic blood pressure, diastolic blood pressure and pulse pressure with annual WML progression. All values mentioned below represent regression

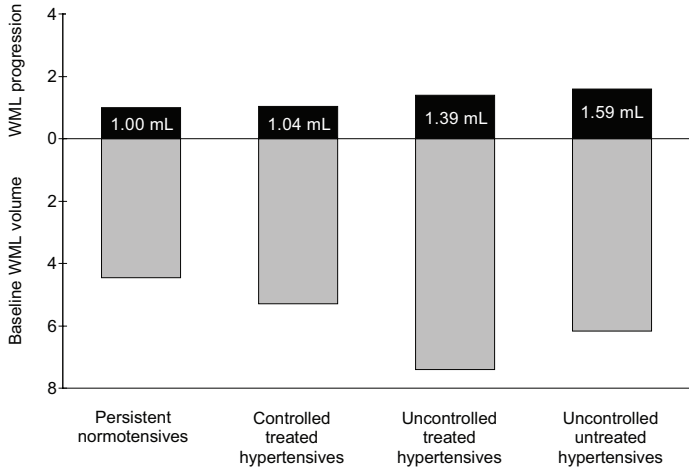

Figure 2. Hypertension treatment and WML progression

This figure shows the mean WML progression in mL (95%-confidence interval) (black bars) on top of the baseline WML volume (grey bars) for four blood pressure categories. Categories were defined as follows based on their mean blood pressure and medication use in the 5 years before the first scan: 1) normotensives: study participants with normal mean blood pressure and receiving no antihypertensive medication during this period (N=255), 2) controlled treated hypertensives: study participants with normal mean blood pressure and receiving antihypertensive medication during this period (N=83), 3) uncontrolled treated hypertensives: study participants with hypertensive mean blood pressure and receiving antihypertensive medication during this period (N=155), and 4) uncontrolled untreated hypertensives: study participants with hypertensive mean blood pressure and receiving no antihypertensive medication during this period (N=172). Hypertensive mean blood pressure was defined as diastolic blood pressure ≥ 90mmHg or systolic blood pressure ≥ 140 mmHg.

coefficients. Both systolic blood pressure and diastolic blood pressure were significantly associated with WML progression, even after adjustment for age, sex, intracranial volume and cardiovascular risk factors (WML progression (95%-confidence interval) per SD increase in systolic blood pressure: 0.08 (0.03; 0.14) mL/year and 0.09 (0.03; 0.15) mL/year per SD increase in diastolic blood pressure. After adjustment for baseline WML volume only the association between systolic blood pressure and WML progression remained statistically significant (0.05 (95%-confidence interval: 0.00; 0.09) mL/year per SD increase in systolic blood pressure, $p < 0.05$). Pulse pressure was also significantly associated with WML progression (0.06 (95%-confidence interval: 0.00; 0.11) mL/year per SD increase in pulse pressure, $p < 0.05$, although this value attenuated and lost statistical significance after adjustment for the presence of hypertension (-0.02 (95%-confidence interval: -0.05; 0.09), mL/year per SD increase in pulse pressure, $p = 0.58$) or adjustment for baseline WML volume (95%-confidence interval: 0.04 (0.00;0.08) mL/year per SD increase in pulse pressure, $p = 0.09$). Addition of quadratic terms of systolic blood pressure, diastolic blood pressure or pulse pressure to any of the models was not significant. After additional adjustments for the presence of cardiovascular disease the effect estimates remained unchanged. Adjustment for *ApoE*-ε4 carriership did not change the effect estimates either. No interaction between

ApoE-ε4 and systolic blood pressure, diastolic blood pressure and pulse pressure was observed (all p-values > 0.20). No differences were observed when body mass index was replaced by the waist/hip ratio as covariate in the analyses.
Figure 2 displays the WML progression on top of the baseline WML volume for nomotensives (N=255), controlled treated hypertensives (N=83), 3) uncontrolled treated hypertensives (N=155), and 4) uncontrolled untreated hypertensives (N=172). We found that the largest amount of WML progression was observed in the uncontrolled untreated hypertensives group. When taking into account the baseline WML load, the WML progression in this group was statistically significant higher than in the uncontrolled treated hypertensives group with a difference (95%-confidence interval) of 0.12 (0.00; 0.23) mL/year, $p < 0.05$. Furthermore, we observed that across people with persistent normal blood pressure, controlled treated hypertension, uncontrolled treated hypertension and uncontrolled untreated hypertension there was a trend of increasing annual WML progression, p=0.01.

DISCUSSION

In this longitudinal MRI study we found that high systolic blood pressure and high diastolic blood pressure were associated with cerebral white matter lesion (WML) progression in the general population. Furthermore, we found that only the assocation with systolic blood pressure remained significant after taking into account the baseline WML load. Finally, we found that WML progression was less in controlled treated hypertensives compared to uncontrolled untreated hypertensives despite a higher baseline WML load.
Major strengths of this study are the measurement of blood pressure before longitudinal MRI-scanning, the quantitatively assessed WML progression and the adjustment for baseline WML volume. This enabled us to address temporality of the relationships, assess WML volume more precisely and evaluate whether any longitudinal associations could be explained by cross-sectional associations between blood pressure and WMLs. Previous studies have addressed one or two of these issues, but none of them addressed all. Another strength is that our population-based setting allows generalizability to a general community-dwelling setting.
A possible limitation of this study is that although the response rate was high, selective drop out could have occurred during the follow-up period. Nevertheless, we believe that if present, this would have led to an underestimation of the results found. Another consideration is that adjustment for baseline WML load is a form of overadjustment because the baseline WML load may be part of the causal chain. However, since the baseline WML load could also act as a confounder, the true effect estimate of the association between blood pressure and WML progression probably lies between the adjusted and unadjusted effect estimates.

Another consideration is that we investigated the 5 year mean blood pressure and not the change in blood pressure over 5 years in relation to WML progresssion. However, blood pressure is highly variable over time and shows considerable regression to the mean.[31] In our study, we therefore decided to use the average over 5 years instead of the change over 5 years. We also note that our study only had two measurements of WML volume, preventing us to assess non-linear trends over time.

High blood pressure has consistently been associated with cross-sectionally measured WML burden [4-13] as well as with longitudinally measured WML progression.[14-15, 17-21] With respect to differences in the associations for systolic blood pressure versus diastolic blood pressure, studies have been inconsistent both for cross-sectionally measured WML burden and longitudinally measured WML progression. Some studies found associations for one of the two, others for both. We found both systolic blood pressure and diastolic blood pressure to be related with WML progression, but only systolic blood pressure to be related with WML progression after taking into acount the already present WML load. Yet, this does not per se imply that systolic blood pressure is more important than diastolic blood pressure in WML progression, because the distribution of systolic blood pressure is possibly more favourable to find associations. We also investigated whether pulse-pressure would give additional information beyond the presence of hypertension. Pulse pressure has been associated with cross-sectional measured WML load in several studies.[6, 32-33] However, we found that the association of pulse pressure with WML progression attenuated and lost statistical significance after taking into acount the presence of hypertension. Perhaps the use of pulse wave velocity or other better indirect measures of arterial stiffness would be more sensitive to pick up an association with WML progression in future studies.

We found that per standard deviation increase in systolic blood pressure blood pressure, WML volume increased with 0.08 mL per year. For diastolic blood pressure this was 0.09 mL per year. This corresponds to 23 % and 25% of the mean annual WML progression in our population, respectively. Several studies found WML progression associated with cognitive decline.[18, 23, 34-35] This implies that high blood pressure could affect cognition via WMLs.

Our finding that people with uncontrolled treated hypertension have significantly less WML progression than people with uncontrolled untreated hypertension implies that treatment of hypertension is important in slowing down WML progression. These results are in line with a recent observational study that found treatment of people with high systolic blood pressure to be related to less WML progression.[21] Recently also randomized controlled trials have been investigating the effect of extra blood pressure lowering treatment compared to standard treatment on WML progression in stroke patients.[36-37] One trial found a protective effect of extra treatment, while the other did not find a difference. Yet, as particiants in these trials were stroke patients, the question remains whether the WMLs in these patients are etiologically similar to the WML disease in the general population.

In conclusion, in this longitudinal MRI study we found that high blood pressure precedes WML progression. Furthermore, we found that hypertension treatment is associated with less WML progression. Our study therefore further establishes high blood pressure as a strong risk factor for WMLs and implies that treatment of hypertension could lead to less white matter lesion progression in the general population. Further studies are needed to assess whether preventing WML development is also relevant to prevent cognitive decline and clinical disease.

REFERENCES

1. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: Systematic review and meta-analysis. *BMJ*. 2010;341:c3666.
2. Xiong YY, Mok V. Age-related white matter changes. *J Aging Res*. 2011;2011:617927.
3. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: A review. *Stroke*. 1997;28:652-659.
4. Fukuda H, Kitani M. Differences between treated and untreated hypertensive subjects in the extent of periventricular hyperintensities observed on brain mri. *Stroke*. 1995;26:1593-1597.
5. Longstreth WT, Jr., Manolio TA, Arnold A, et al. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The cardiovascular health study. *Stroke*. 1996;27:1274-1282.
6. Liao D, Cooper L, Cai J, Toole J, et al. The prevalence and severity of white matter lesions, their relationship with age, ethnicity, gender, and cardiovascular disease risk factors: The aric study. *Neuroepidemiology*. 1997;16:149-162.
7. Swan GE, DeCarli C, Miller BL, et al. Association of midlife blood pressure to late-life cognitive decline and brain morphology. *Neurology*. 1998;51:986-993.
8. de Leeuw FE, de Groot JC, Oudkerk M, et al. A follow-up study of blood pressure and cerebral white matter lesions. *Ann Neurol*. 1999;46:827-833.
9. Dufouil C, de Kersaint-Gilly A, Besancon V, et al. Longitudinal study of blood pressure and white matter hyperintensities: The eva mri cohort. *Neurology*. 2001;56:921-926.
10. van Dijk EJ, Breteler MM, Schmidt R, et al. The association between blood pressure, hypertension, and cerebral white matter lesions: Cardiovascular determinants of dementia study. *Hypertension*. 2004;44:625-630.
11. Shrestha I, Takahashi T, Nomura E, et al. Association between central systolic blood pressure, white matter lesions in cerebral mri and carotid atherosclerosis. *Hypertens Res*. 2009;32:869-874.
12. Vuorinen M, Solomon A, Rovio S, et al. Changes in vascular risk factors from midlife to late life and white matter lesions: A 20-year follow-up study. *Dement Geriatr Cogn Disord*. 2011;31:119-125.
13. Carmelli D, Swan GE, Reed T, Wolf PA, Miller BL, DeCarli C. Midlife cardiovascular risk factors and brain morphology in identical older male twins. *Neurology*. 1999;52:1119-1124.
14. Veldink JH, Scheltens P, Jonker C, Launer LJ. Progression of cerebral white matter hyperintensities on mri is related to diastolic blood pressure. *Neurology*. 1998;51:319-320.
15. Schmidt R, Enzinger C, Ropele S, Schmidt H, Fazekas F, Austrian Stroke Prevention S. Progression of cerebral white matter lesions: 6-year results of the austrian stroke prevention study. *Lancet*. 2003;361:2046-2048.
16. Sachdev P, Wen W, Chen X, Brodaty H. Progression of white matter hyperintensities in elderly individuals over 3 years. *Neurology*. 2007;68:214-222.
17. Firbank MJ, Wiseman RM, Burton EJ, Saxby BK, O'Brien JT, Ford GA. Brain atrophy and white matter hyperintensity change in older adults and relationship to blood pressure. Brain atrophy, wmh change and blood pressure. *J Neurol*. 2007;254:713-721.
18. van Dijk EJ, Prins ND, Vrooman HA, Hofman A, Koudstaal PJ, Breteler MM. Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences: Rotterdam scan study. *Stroke*. 2008;39:2712-2719.
19. Gottesman RF, Coresh J, Catellier DJ, et al. Blood pressure and white-matter disease progression in a biethnic cohort: Atherosclerosis risk in communities (aric) study. *Stroke*. 2010;41:3-8.
20. Debette S, Seshadri S, Beiser A, et al. Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. *Neurology*. 2011;77:461-468.
21. Godin O, Tzourio C, Maillard P, Mazoyer B, Dufouil C. Antihypertensive treatment and change in blood pressure are associated with the progression of white matter lesion volumes: The three-city (3c)-dijon magnetic resonance imaging study. *Circulation*. 2011;123:266-273.
22. Gouw AA, van der Flier WM, Fazekas F, et al. Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: The leukoaraiosis and disability study. *Stroke*. 2008;39:1414-1420.

23. White WB, Wolfson L, Wakefield DB, et al. Average daily blood pressure, not office blood pressure, is associated with progression of cerebrovascular disease and cognitive decline in older people. *Circulation*. 2011;124:2312-2319.
24. Hofman A, van Duijn CM, Franco OH, et al. The rotterdam study: 2012 objectives and design update. *Eur J Epidemiol*. 2011;26:657-686.
25. Ikram MA, van der Lugt A, Niessen WJ, et al. The rotterdam scan study: Design and update up to 2012. *Eur J Epidemiol*. 2011;26:811-24.
26. Vrooman HA, Cocosco CA, van der Lijn F, et al. Multi-spectral brain tissue segmentation using automatically trained k-nearest-neighbor classification. *Neuroimage*. 2007;37:71-81.
27. de Boer R, Vrooman HA, van der Lijn F, et al. White matter lesion extension to automatic brain tissue segmentation on mri. *Neuroimage*. 2009;45:1151-1161.
28. Vernooij MW, Ikram MA, Tanghe HL, et al. Incidental findings on brain mri in the general population. *N Engl J Med*. 2007;357:1821-1828.
29. Leening MJ, Kavousi M, Heeringa J, et al. Methods of data collection and definitions of cardiac outcomes in the rotterdam study. *Eur J Epidemiol*. 2012;27:173-185.
30. Slooter AJ, Cruts M, Kalmijn S, et al. Risk estimates of dementia by apolipoprotein e genotypes from a population-based incidence study: The rotterdam study. *Arch Neurol*. 1998;55:964-968.
31. Gordon T, Sorlie P, Kannel WB. Problems in the assessment of blood pressure: The framingham study. *International journal of epidemiology*. 1976;5:327-334.
32. Kim CK, Lee SH, Kim BJ, Ryu WS, Yoon BW. Age-independent association of pulse pressure with cerebral white matter lesions in asymptomatic elderly individuals. *J Hypertens*. 2011;29:325-329.
33. Henskens LH, Kroon AA, van Oostenbrugge RJ, et al. Increased aortic pulse wave velocity is associated with silent cerebral small-vessel disease in hypertensive patients. *Hypertension*. 2008;52:1120-1126.
34. Schmidt R, Ropele S, Enzinger C, et al White matter lesion progression, brain atrophy, and cognitive decline: The austrian stroke prevention study. *Ann Neurol*. 2005;58:610-616.
35. Longstreth WT, Jr., Arnold AM, Beauchamp NJ, et al. Incidence, manifestations, and predictors of worsening white matter on serial cranial magnetic resonance imaging in the elderly: The cardiovascular health study. *Stroke*. 2005;36:56-61.
36. Dufouil C, Chalmers J, Coskun O, et al. Effects of blood pressure lowering on cerebral white matter hyperintensities in patients with stroke: The progress (perindopril protection against recurrent stroke study) magnetic resonance imaging substudy. *Circulation*. 2005;112:1644-1650.
37. Weber R, Weimar C, Blatchford J, et al. Telmisartan on top of antihypertensive treatment does not prevent progression of cerebral white matter lesions in the prevention regimen for effectively avoiding second strokes (profess) mri substudy. *Stroke*. 2012;43:2336-42.

Chapter 5

General discussion

In this thesis I investigated determinants of subclinical brain disease in the middle-aged and elderly general population, thereby focusing on three main domains: 1) developments of novel methodology to study the etiology of subclinical brain disease, 2) genetic determinants of subclinical brain disease, and 3) modifiable determinants of subclinical brain disease. In general there was a focus on subclinical *vascular* brain disease. In this chapter I will review my main findings, address methodological considerations and discuss possible implications and future directions.

MAIN FINDINGS

Novel methodology to assess subclinical brain disease

The development of novel techniques to detect and quantify brain pathology in vivo is essential for brain research and for research on subclinical brain disease in particular. Because the earliest brain pathology is often subtle, sensitive methods are needed to pick up small abnormalities. For imaging, this means that computer-aided image processing is required to pick up abnormalities that are not visible to the human eye. In addition, because large sample sizes are needed to gain enough statistical power, computer technology is needed to handle large volumes of data in a consistent and efficient manner.

It has been hypothesized that causes and consequences of white matter lesions may differ depending on the region in which they occur.[1-4] However, local white matter lesion volume has mostly been quantified with methods that rely on the distinction between lesions in a periventricular region close to the ventricles and a subcortical zone further away. Yet, it has been suggested that such a distinction may be arbitrary.[5] We therefore developed a method to segment and measure white matter lesion volume in 43 regions defined by orientation and distance to the ventricles. The potential of the method was demonstrated by analyzing the effect of blood pressure on the regional white matter lesion volume in 490 elderly people taken from the Rotterdam Study. The main finding was that high blood pressure was primarily associated with lesion load in the vascular watershed area that forms the border between the periventricular and subcortical regions. This supports the current concept that white matter lesions are the result of ischemic injury. Furthermore, it shows that a method that only distinguishes periventricular lesions and subcortical lesions is too crude to investigate whether lesions in different areas have different causes and consequences.
It is unknown whether white matter lesions develop abruptly in previously normal brain areas, or whether tissue changes are already present before white matter lesions become apparent on an MRI. Knowledge about this is important to identify in which persons and at what moment preventive measures should be installed. Previously it was not possible to

study this, but recent advances in imaging enable the detection of these subtle changes. In a sample of 689 people from the Rotterdam Study we performed two MRI scans 3.5 years apart and studied whether new white matter lesions on the second MRI scan were preceded by subtle abnormalities in the same region on the first MRI scan. To detect these subtle changes we used diffusion tensor imaging measures and the FLAIR-intensity value. We performed regional matching and a voxel-based approach to take into account the lesion location of measurements in the white matter. We found that subtle changes in the white matter are indeed present before white matter lesions develop and that this holds true for both newly occurring lesions as well as growing lesions. Our results are in line with findings from a cross-sectional study that found subtle white matter abnormalities in the area surrounding white matter lesions.[6] These results show that white matter lesions are only the tip of the iceberg in white matter pathology, and suggest that white matter lesion development may be predicted years before it becomes apparent with conventional imaging techniques.

Genetic determinants of subclinical brain disease

Genetic determinants of subclinical brain disease can point to pathophysiological pathways that can be modified in order to prevent disease. Yet, the genetics of subclinical brain disease are a virtually unexplored area. For my thesis, I focused on four projects in this regard.

Although white matter lesions have been shown to be highly heritable, a genetic locus for white matter lesions in the general population was identified only recently.[7] Replication of such a finding in an independent sample contributes to its credibility. We therefore investigated whether this locus was associated with white matter lesions in an independent sample of 1,677 people from the Rotterdam Study. We found that this locus was indeed associated with white matter lesions. Thereby we further established the role of this locus in white matter lesions. Furthermore, this finding contributes to the generalizability of the results, since our study population was relatively young compared with the populations of the discovery study (mean age 56 years versus mean age older than 65 years). In addition, we found that this genetic locus was associated with white matter lesions, independent of cardiovascular risk factors such as high blood pressure. Interestingly, the Chr17q25 locus has now also been replicated in Japanese people[8] and the locus has been found to also be associated with white matter lesion volume in ischemic stroke patients.[9]

Following the first discovery of this new locus for white matter lesions, we are leading an international effort to identify additional genetic loci. We therefore increased the sample size of the original discovery sample by collaborating with studies from France, Germany, Great Britain, Iceland, Singapore, and the United States, and imputed our data to the *1000*

Genomes reference panel to gain more statistical power.[10] In total, we reached a sample size of 21,079 and included people from European descent, Afro-Americans, Hispanics, and Asians. This resulted in the discovery of four novel loci and several suggestive loci that almost reached statistical significance. This project is still ongoing but shows great promise in revealing new pathways for white matter lesion development.

In another project we set out to investigate the influence of previously identified genetic loci on cognition. In recent years, genome-wide association studies had identified several new genes for Alzheimer's disease in addition to the already known *ApoE* gene.[11-15] To study whether these genes already have early effects, we investigated whether these novel genes jointly affected cognitive function in 5,171 non-demented people (age 45-99 year). To capture the joint effect of these genes, we constructed genetic risk scores based on the number and type of risk alleles a person carries. We found that the novel genes only had a very subtle effect on memory. Furthermore, these genes jointly did not improve the prediction of Alzheimer's disease beyond *ApoE*. Probably these genes have very subtle effects and are overshadowed by other factors. Recently a study found that prediction may improve if interactions in the statistical model are taken into account (doi:10.1016/j.biopsych.2013.07.008). Prediction is also expected to improve by adding more genes to the model, which may derive from new meta-analyses of genome-wide association studies.

Genetic loci that have been identified for a specific trait can also be used to study the relation between this trait and a more downstream trait. We applied this concept in a study of the relation of blood pressure to the retinal and cerebral circulation. Retinal arteriolar diameters served as a measure of the condition of the retinal circulation and the total cerebral blood flow as a measure of the condition of the cerebral circulation. We found that genetic loci for blood pressure were associated with the arteriolar diameters in the retina, but not with cerebral blood flow. We replicated this finding in an independent cohort.[16] This suggests that the relation between blood pressure and the retinal circulation has a genetic basis. The absence of an association of the blood pressure loci and cerebral blood flow may be due to the fact that the cerebral blood flow is more influenced by other factors than blood pressure, e.g., oxygen pressure or metabolic demand.

Modifiable determinants of subclinical brain disease

To prevent or slow down subclinical brain disease, determinants need to be identified that can be modified. In this part of my thesis, I sought to identify and investigate the role of possible modifiable factors that influence aging of the brain.

First, we investigated whether the incidence of dementia has decreased over the last decades. Assuming that the genetic make-up of the study population has remained

relatively constant over the years, this throws light upon the influence of environmental factors on the condition of the brain. To investigate this, we compared the incidence of dementia in a cohort started in 1990 with the dementia incidence in a cohort that started in 2000. Interestingly, we found that the incidence of dementia had decreased over the past decades. Furthermore, we found that this decrease was accompanied by a decrease in brain pathology on MRI. This suggests that people's brains have been exposed to less vulnerable environmental exposures. This finding is in line with a recent report that nonagenarians now perform cognitively better than ten years ago in Denmark (http://dx.doi.org/10.1016/S0140-6736(13)60777-1) and with the results from a study in Sweden that also found evidence for a decrease in the incidence of dementia.[17] However, because the aging of the population still puts many people at risk, the coming dementia epidemic will remain substantial. Yet, these finding are encouraging and imply that there may be more to win by modifying the exposure to deleterious factors.

Because a substantial part of the subclinical brain disease is related to vascular disease, much may be gained with modifying the exposure to vascular risk factors. Although many vascular risk factors have been identified, the identification of novel vascular risk factors may further improve disease prevention. Uric acid has recently been reappraised as an independent cardiovascular factor and levels of uric acid are modifiable. However, how levels of uric acid relate to brain atrophy and cognition was not known. In a population-based sample of 814 people without stroke or dementia we studied this and found that high uric acid levels were associated with white matter atrophy and worse cognitive function, which was independent of potential confounders. In addition, we found that the association was mainly driven by people with hyperuricemia and not by people with normal uric acid levels. Our study therefore implies that prevention or treatment of hyperuricemia may be beneficial in the prevention of subclinical brain disease.

Besides the identification of novel vascular risk factors, benefits may be gained from the critical appraisal of the role of established cardiovascular risk factors. High blood pressure is considered to be one of the most important risk factors for white matter lesions. Yet, essential information is missing with regard to causal inference. Previous studies have largely been unable to unequivocally demonstrate that novel white matter lesions are indeed preceded by high blood pressure because of the applied study designs. We therefore performed a population-based longitudinal MRI study in which we measured blood pressure in a five-year period before the measurement window for novel white matter lesions. In addition, we studied the role of anti-hypertensive medication on white matter lesion progression. We found that blood pressure indeed precedes the development of white matter lesions and that this was independent of potential confounders and the pre-existing white matter lesion

load. Furthermore we found that treatment of hypertension was related to less progression. Our study therefore adds to the evidence suggesting that antihypertensive treatment may be beneficial in the prevention of white matter lesions.

METHODOLOGICAL CONSIDERATIONS

In this section, I discuss several methodological considerations related to the study methods applied in this thesis. These considerations are inherent to study design or applied technologies, and are therefore grouped accordingly.

Study design

All studies described in this thesis are wholly or for a large part based on data from the Rotterdam Study, and more in particularly, the Rotterdam Scan Study. The Rotterdam Study is a population-based prospective cohort study that is designed to investigate the causes of diseases in the middle-aged and elderly.[18] It consists of three cohorts: Rotterdam Study I (baseline in 1990, age ≥ 55 years), Rotterdam Study II (baseline in 2000, age ≥ 55 years) and Rotterdam Study III (baseline in 2008, age ≥ 45 years). In total, 14,926 people are followed-up and receive extensive examination rounds every three to four years. The Rotterdam Scan Study is nested in this study and includes all the people within the Rotterdam Study that received a brain MRI (at present > 5,000 people).[19] Although response rates were high and attrition rates low, it may be that the population is slightly healthier than the general population. We however believe that any potential selection effect was small and that, if present, has led to an underestimation of the associations found. The database of the Rotterdam Study is linked to the records of the general practitioners. Therefore, information on clinical disease that was not reported by the participants (e.g., stroke, dementia) was also registered.

Putative confounders were assessed at each visit to be able to deal with confounding. Although this assessment was comprehensive, it may be possible that some residual confounding persisted.

For the meta-analysis of genome-wide associations on white matter lesions we sought to include only population-based samples. A major advantage of this approach is the generalizability to the general population. However, population-based samples may show a lower variability of white matter lesions than clinical samples and therefore may have lower statistical power. Furthermore, clinical samples may also capture people that tend to avoid participating in intensive investigation rounds of population-based studies.

Imaging

Because imaging is highly suited to measure subclinical brain disease non-invasively in vivo, the work in my thesis relies heavily on imaging methods and imaging processing methods. Developments in imaging hardware and software are fast, which renders today's methods easily outdated. For example, in the Rotterdam Scan Study, we used a 1.5 T MRI for the measurements of white matter lesions, white matter structural integrity, brain atrophy and infarcts. At present, MRI scanners with higher field strength are available that are probably more sensitive to pick up pathology due to higher signal-to-noise ratio and/or higher spatial resolution. However, in our study design, there is a trade-off between having the newest scan techniques and the usefulness of the scans for research questions. When serial MRI scans are performed over years as in the Rotterdam Scan Study, it is highly desirable to make these measurements on the same scanner to ensure consistency in measurement accuracy. This limits biases in the measurements of, for example, brain atrophy. Another imaging-related issue is how to reliably assess brain tissue volumes – and in particular changes in volume - on MRI. Traditionally, visual rating scales were used for white matter lesion volume or brain atrophy. This is not only very time-consuming, but also has a considerable interrater and intrarater variability. Besides, it only resulted in a score and not in the exact volume of pathology and is also therefore less sensitive to subtle changes. In recent years, automated methods were developed that can avoid these problems by segmenting brain tissue and quantifying volume in a precise and consistent manner. In the Rotterdam Study we used a validated automated method where possible and performed visual inspection to remove possible errors.

Another issue is the comparability of imaging studies. Studies now use different scanners, with different scanner settings and different pathology measurement methods. Therefore it may be more difficult to replicate findings in an independent sample. In addition, statistical power to find associations with meta-analyses will be reduced. As multi-center studies and consortium studies are emerging in the field, this is becoming increasingly important. On the other hand, if an association is present in multiple studies, despite the heterogeneity, it is more likely that this finding is related to the pathology that was intended to measure than that it is related to a scanning or measurement artifact.

Cognition

The condition of the brain can also be derived from its functioning, for example cognitive performance. Within the Rotterdam Study we use a standardized test battery to measure cognition that includes tests to capture memory function, executive function and information processing speed. To gain more robust measures of cognition we constructed compound scores from these separate tests. Like all other measurements, these cognitive tests were performed without knowledge of the variables of interest to prevent information

bias. A potential limitation of the used tests is that they may not be able to detect subtle abnormalities. An issue that may have caused noise is the possibility of a learning effect when people have had the same cognitive tests before. Another limitation is that compound scores from different cognitive domains may share the same tests. Recently, a method has been introduced to better capture cognitive tests in a summary measure, called the G-factor.[20] In addition, the G-factor may be more robust than previously used summary measures.

Because the focus of my thesis was to study subclinical disease, it was important to exclude people with dementia. Therefore if people scored low on specific cognitive tests, they were screened for dementia by a research physician in a more extensive questionnaire. Furthermore, because of linkage with the general practitioner's records, additional dementia cases were picked up. Possible dementia cases were finally evaluated in a panel containing an experienced neurologist to decide whether a person had dementia. Yet, it may be possible that people with dementia may have been included in the samples to study subclinical brain disease, which may have led to an overestimation of the found associations.

Genetics

For this thesis, I used genome-wide genetic data to discover, to confirm and to investigate the potential effects of genetic loci on subclinical brain disease. For the discovery of genetic loci in genome-wide association studies very large sample sizes are needed because millions of tests are performed simultaneously, which makes the chance of false-positive results high. In addition, sample sizes need to be large because genetic loci tend to have subtle effects. Studies need to collaborate with each other and pool or meta-analyze their results to reach the desired large sample sizes. Although there is a substantial statistical power loss because studies generally differ considerably in the assessment of exposure and outcome, this can be overcome by increasing sample size. It is also important which reference panel is used to impute non-genotyped DNA-variants. This will also determine your power and your ability to find rare variants. Traditionally *HapMap* was used which was well suited for identifying variants with a minor allele frequency of 5 % and higher,[21] while now *1000 Genomes* is increasingly being used and is well suited to identify variants with a minor allele frequency of higher than 1 %.[10] The Dutch population now has its own reference panel since very recently: *GoNL*, which can be considered as the *1000 Genomes* of the Netherlands.[22] This will improve imputations in Dutch studies and thus lead to more sensitivity to detect rare variants. Furthermore, it will also enable the study of population-specific variants.

Apart from the methods to assess the genotypes, it should be noted that the DNA variants that are identified in genome-wide association studies are not per se the causative variants of a trait. Because not all variants are captured in traditional genome-wide association, it is likely that the identified variant is only in close relation with the causal variant. This causal

variant can lie close to the identified variant, but could also be further away. Genome or exome sequencing and functional studies may help in further pinpointing of the causal variants.
When a genetic variant is found, traditionally replication should be sought. This already stems from the era before genome-wide association studies, in which potential genetic loci were often identified, but replication was unsuccessful. Also in this thesis, we replicated the genetic locus that was identified in the first genome-wide association study on white matter lesions. Such a replication further establishes the found association, especially if this is in a population is of different ethnicity.
As most effects of common variants on diseases are small, approaches that combine effects of genetic loci into genetic risk scores or genetic burden scores can increase statistical power to assess associations. Genetic risk scores and genetic burden scores are in essence the same, except that the risk score refers to a dichotomous trait and the burden score to continuous trait. These genetic scores may help in the prediction of disease or pathology, but also in etiologic / pathophysiologic research, because one can study how the genetic variants of one trait influence another trait. While it is also possible to study every single variant separately, genetic risks score can avoid the problem of multiple-testing and its related false-positive results. Furthermore, the genetic variants together can have synergistic effects, which will be missed when every genetic variant is studied separately. A disadvantage of a genetic score is that an effect of one genetic variant can be missed, because it is attenuated by the other variants in the score.

CLINICAL IMPLICATIONS

The focus of this thesis was to identify and investigate the role determinants of subclinical age-related brain disease in order to find pathophysiological pathways that can be targeted for the early prevention or early treatment of clinical diseases such as dementia and stroke. Further research is needed to further explore the clinical usefulness of these determinants. Eventually, intervention studies are needed to test whether removal or reduction of the exposure to these determinants leads to a better clinical outcome. Nevertheless, there are important conclusions to be drawn from the research of this manuscript.

First, we have demonstrated the potential of imaging and image processing methods to better quantify subclinical brain disease. This may lead to a more complete assessment of the condition of the brain on MRI. These methods may aid radiologists and neurologists in better quantifying the extent of a patient's subclinical brain disease and may make descriptions in words superfluous. This may improve the identification of people at risk and

the people who may benefit from a specific treatment.
Second, we have confirmed, identified, and investigated the effect of novel genetic variants on subclinical brain disease. This knowledge provides insights in etiological/pathophysiological pathways of neurological disease that may be targeted for prevention and may help in identifying people at risk.
Third, we showed that the trend is that the risk of dementia has slightly decreased over the last decades. Nevertheless, due to the aging of populations, the number of people that will be affected by dementia will still be huge, necessitating relevant markers to intervene upon. The fact that the decrease in dementia incidence was accompanied by less vascular brain damage and more treatment of cardiovascular risk factors suggests this may be caused by better control of vascular disease, and thus points towards vascular factors as potentially modifiable factors. Our studies on blood pressure and uric acid support this. The knowledge gained from the study of vascular risk factors for subclinical brain disease in this thesis may further help in this regard, and makes a strong case for further research on potentially modifiable factors.

SUGGESTIONS FOR FUTURE RESEARCH

Future research needs to be directed at further exploration of determinants of subclinical brain disease, the development of novel methods to quantify subclinical brain disease, and the further assessment of clinical implications. Imaging, cognition and genetics provide important tools in this regard, and therefore suggestions for future research are discussed for each of them.

Imaging

Although subclinical brain disease has been studied for decades, we have only recently found out that what is visible to the human eye on an MRI scan is only the tip of the iceberg of the true pathology. Advances in imaging are excellently suited to further explore this, as they can be applied non-invasively and at low cost. We for example showed that with improvements in image processing (measuring lesion location) and imaging technique (diffusion tensor imaging) much can be gained. Further developments are to be expected in both fields. Instead of investigating lesions as a whole, voxel-based approaches can be used, and instead of investigating global microstructure, tract-specific microstructure can be studied. All these developments have implications on how we approach health versus disease in studies and trials. This will be more a continuum instead of dichotomous condition of presence and absence. These measures will therefore become increasingly more important in the inclusion/exclusion of subjects for trials and the monitoring of therapy. Furthermore,

they may help in the early identification of people at risk and the prognosis assessment in people that already have dementia or stroke. Challenges will be faced in the harmonization of protocols and processing algorithms across studies.

Cognition

Like brain imaging, cognitive research may also greatly benefit from advances in digital technology. Traditionally, cognitive tests have been performed by interview and questionnaire with pen and paper, but this strongly limits the amount of data that can be extracted from the results. If for example the drawing of a clock could be performed on a touch screen then not only the end result can be registered, but also the drawing speed, the fluency, the symmetry, and the extent of tremor and dysmetry. Furthermore, this not only provides information on cognition, but also on motor skills. The cognitive component of a test can therefore be better distinguished from the motor component. Furthermore, because the performance can be analyzed on a continuous scale, more statistical power is to be expected with such measurements and therefore subtle abnormalities could be detected earlier. This may help in the early detection of dementia or stroke. In addition, such a precise marker may well be used at the clinical department to assess and monitor a patient's cognitive function during admission or when treated.

Genetics

Thanks to genome-wide association data we have confirmed and identified several genetic loci for subclinical brain disease. Yet, for pinpointing the exact causal polymorphisms further studies are needed. Sequencing and experimental studies may be helpful in this. In addition, the effect of the identified variants on clinical disease can be studied and can be used in models to predict for example dementia or stroke. To also identify very rare variants (minor allele frequency < 1%), sample sizes in the studies should be increased. Another strategy to improve the ability of finding variants is to perform genome-wide association studies in studies with a trio-design, i.e., including the parents of an individual. This way de-novo mutations can also be found. Besides, much can be gained from research that focuses on gene-gene interactions and gene-environment interactions. Trials in future may increasingly take into account genetic profiles. This may for example lead to preventive strategies for stroke and dementia that are adjusted to the genetic profile of a person.

To gain more insight into the etiology and pathophysiology of disease, parallels need to be drawn between findings for one trait and findings for another trait. They can reveal common pathways and intermediate pathways of diseases. The use of genetic risk scores and genetic burden scores can help in this, as we have shown in this thesis. On the other hand, multiple related traits can be combined into one compound trait. This could increase the likelihood of finding new loci if a common etiologic pathway is suspected. Alternatively, a meta-analysis

could be performed of meta-analyses of different traits. Furthermore, parallels need to be drawn between disease in the young and disease in the old. The development of the brain at young age, already starting at conception, is crucial for later brain function, and minor abnormalities at a young age may later aggravate and lead to dementia in old age. To the extreme, it even might be possible that the genetics of the mother are already important for brain development of the child. A certain genetic make-up which makes the mother susceptible to high glucose levels may for example influence brain development. Therefore, it would also be of interest to perform genome-wide associations on the maternal genome for a trait in the child, which could be done in trio-designs.
At present most genetic studies are performed in people from European descent. This limits the generalizability of findings to the majority of the world's population. Fortunately, studies are increasingly including more and more people from non-European ethnicities.

CONCLUDING REMARKS

In this chapter I sought to put the main findings of my thesis into perspective and to discuss methodological considerations, clinical implications and suggestions for future research. I felt happy to work on this project and to combine two rapidly developing and promising fields of science: genetics and imaging. I think that the best can be found where different fields of science combine their powers holds true for every topic in science.

REFERENCES

1. de Groot JC, de Leeuw FE, Oudkerk M, Hofman A, Jolles J, Breteler MM. Cerebral white matter lesions and depressive symptoms in elderly adults. *Archives of general psychiatry.* Nov 2000;57(11):1071-1076.
2. de Groot JC, de Leeuw FE, Oudkerk M, et al. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. *Annals of neurology.* Feb 2000;47(2):145-151.
3. Gouw AA, Seewann A, van der Flier WM, et al. Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. *Journal of neurology, neurosurgery, and psychiatry.* Feb 2011;82(2):126-134. Kim KW, MacFall JR, Payne ME. Classification of white matter lesions on magnetic resonance imaging in elderly persons. *Biological psychiatry.* Aug 15 2008;64(4):273-280.
5. DeCarli C, Fletcher E, Ramey V, Harvey D, Jagust WJ. Anatomical mapping of white matter hyperintensities (WMH): exploring the relationships between periventricular WMH, deep WMH, and total WMH burden. *Stroke; a journal of cerebral circulation.* Jan 2005;36(1):50-55.
6. Maillard P, Fletcher E, Harvey D, et al. White matter hyperintensity penumbra. *Stroke; a journal of cerebral circulation.* Jul 2011;42(7):1917-1922.
7. Fornage M, Debette S, Bis JC, et al. Genome-wide association studies of cerebral white matter lesion burden: the CHARGE consortium. *Annals of neurology.* Jun 2011;69(6):928-939.
8. Tabara Y, Igase M, Okada Y, et al. Association of Chr17q25 with cerebral white matter hyperintensities and cognitive impairment: the J-SHIPP study. *European journal of neurology : the official journal of the European Federation of Neurological Societies.* May 2013;20(5):860-862.
9. Adib-Samii P, Rost N, Traylor M, et al. 17q25 Locus is associated with white matter hyperintensity volume in ischemic stroke, but not with lacunar stroke status. *Stroke; a journal of cerebral circulation.* Jun 2013;44(6):1609-1615.
10. Genomes Project C, Abecasis GR, Auton A, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature.* Nov 1 2012;491(7422):56-65.
11. Lambert JC, Heath S, Even G, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet.* Oct 2009;41(10):1094-1099.
12. Harold D, Abraham R, Hollingworth P, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet.* Oct 2009;41(10):1088-1093.
13. Seshadri S, Fitzpatrick AL, Ikram MA, et al. Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA : the journal of the American Medical Association.* May 12 2010;303(18):1832-1840.
14. Naj AC, Jun G, Beecham GW, et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet.* May 2011;43(5):436-441.
15. Hollingworth P, Harold D, Sims R, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet.* May 2011;43(5):429-435.
16. Harris TB, Launer LJ, Eiriksdottir G, et al. Age, Gene/Environment Susceptibility-Reykjavik Study: multidisciplinary applied phenomics. *American journal of epidemiology.* May 1 2007;165(9):1076-1087.
17. Qiu C, von Strauss E, Backman L, Winblad B, Fratiglioni L. Twenty-year changes in dementia occurrence suggest decreasing incidence in central Stockholm, Sweden. *Neurology.* May 14 2013;80(20):1888-1894.
18. Hofman A, van Duijn CM, Franco OH, et al. The Rotterdam Study: 2012 objectives and design update. *Eur J Epidemiol.* Aug 30 2011.
19. Ikram MA, van der Lugt A, Niessen WJ, et al. The Rotterdam Scan Study: design and update up to 2012. *Eur J Epidemiol.* Oct 16 2011.
20. Jensen RA. *The g factor: The Science of Mental Ability.* Praeger; 1998.
21. International HapMap C. The International HapMap Project. *Nature.* Dec 18 2003;426(6968):789-796.
22. Boomsma DI, Wijmenga C, Slagboom EP, et al. The Genome of the Netherlands: design, and project goals. *European journal of human genetics : EJHG.* May 29 2013.

Chapter 6

Summary / Samenvatting

Chapter 7

Dankwoord

PhD Portfolio

List of Publications

About the Author

DANKWOORD

De afgelopen jaren heb ik met plezier gewerkt aan het werk beschreven in dit proefschrift. Al het werk heb ik niet alleen verricht en op deze plaats wil ik velen bedanken.

Ten eerste wil ik mijn promotoren prof.dr. Hofman en prof.dr. Van der Lugt bedanken. Beste Bert, jouw enthousiasme en methodologisch inzicht hebben mij geinspireerd en jouw toegankelijkheid heb ik gewaardeerd. Beste Aad, jouw radiologische expertise, prettige manier van communiceren en goede, altijd to-the-point-commentaar maakten het fijn om met jou te werken. Daarnaast ben je inspirerend geweest voor mijn keuze om radioloog te worden.

Eveneens wil ik mijn co-promoteren dr. Ikram en dr. Vernooij bedanken. Ik denk dat ik geen betere co-promoteren had kunnen hebben. Jullie positieve mindset, slimheid, rapheid en toegankelijkheid zijn van enorme waarde geweest. Beste Arfan, ik vond het altijd fijn en gezellig met jou te werken. Ik vond de gezamenlijke congresbezoeken erg leuk. Beste Meike, niet alleen ben je een geweldige co-promoter, ik heb gezien dat je ook een goede people-manager bent.

Prof.dr. Breteler, beste Monique, jij hebt mij de gelegenheid gegeven om onderzoek te doen binnen de Rotterdam Study, eerst als masterstudent en later als promovendus. Van begin af aan voelde ik dat het goed zat. Jouw benadering van wetenschap en kunde van presenteren in woord en geschrift zijn inspirerend voor mij geweest en denk ik ook voor vele anderen. Ik wil jou daarvoor bedanken.

Prof.dr. Van Duijn en prof.dr. Biessels wil ik graag bedanken voor zitting te nemen in de leescommissie en de bereidheid te opponeren tijdens de openbare verdediging van mijn proefschrift. Prof.dr. Van Duijn wil ik ook bedanken voor haar wetenschappelijke feedback op mijn papers met betrekking tot de genetica. I would like to thank Dr. Michael O'Sullivan for serving on my thesis committee and for travelling to the Netherlands for my public defense. Daarnaast wil ik prof.dr. De Leeuw, prof.dr. Niessen en prof.dr. Sijbrands bedanken voor zitting te nemen in mijn promotiecomissie. Prof.dr. Niessen wil ik ook bedanken voor zijn expertise en feedback op het gebied van imaging.

Mijn dank gaat uit naar prof.dr. Krestin voor het geven van de gelegenheid om radiologisch onderzoek te doen en voor de mogelijkheid om mijn opleiding tot radioloog te volgen in het Erasmus MC. Dr. Van Lankeren dank ik voor het geven van de ruimte en tijd voor de laatste loodjes van mijn promotie tijdens mijn opleiding Radiologie.
Alle co-auteurs van de verschillende hoofdstukken wil ik bedanken voor hun bijdragen. Een

aantal mensen wil ik in het bijzonder bedanken. Dr. Myriam Fornage, I would like to thank you for supervising the work on the manuscript about the genome-wide association study on white matter lesions. Your expertise and feedback were of great value. I also would like to thank the other NeuroCHARGE members for their kind collaboration: Prof.Dr. Seshadri, Prof. Dr. Longstreth, Dr. Bis, ms. and mr. Prof.Dr. Schmidt and Prof.Dr. Launer. Bedankt Marius de Groot voor de fijne samenwerking met betrekking tot het "deltawml-paper". Wat geweldig dat we hier ook nog een prijs voor hebben gekregen! Renske de Boer, bedankt voor je geweldige wittestoflesie-segmentatiemethode! Hieab Adams, we hebben veel samengewerkt zowel met scanklusjes als met analyses. Ik vond het altijd erg gezellig. Ik denk dat je het ver gaat schoppen. Daniel Bos, inmiddels dr. Bos, ik heb de samenwerking met jou erg gewaardeerd. Marielle Poels, ook inmiddels dr. Poels, mijn RSS-partner van het eerste uur, jij ook bedankt voor de fijne samenwerking. Dr. Abbas Dehghan, wil ik graag bedanken voor het mij wegwijs maken in de wereld van de GWAS.

Mijn speciale dank gaat uit naar alle duizenden deelnemers van de Rotterdam Study (ERGO). Dankzij jullie kunnen wij heel mooi onderzoek doen en publiceren in vooraanstaande wetenschappelijke tijdschriften. Ik wil graag ook alle medewerkers bedanken van het ERGO-onderzoekscentrum. Ik vond het fijn om met jullie te werken. In het bijzonder wil ik dr. Jan Heeringa bedanken voor de coördinatie van het ERGO-onderzoek en Lydia, Pauli, Marja, Karin en Charlotte voor hun werk met betrekking tot het MRI-onderzoek. Ook de dames van het secretariaat Hetty, Jacqueline, Erica, Gabriëlle, Esther en Marion wil ik bedanken voor hun vriendelijkheid en organisatie. Ik ben de dame en heren van de automatisering en datamanagement Jolande, Nano, René, Frank, René en Eric dankbaar voor het altijd klaar staan om te helpen bij een computerprobleem, dataprobleem of het opvragen van gegevens.

Mijn collega onderzoekers van de neuro-epidemiologie Ana, Daniel, Dymph, Elisabeth, Elizabeth, Evert, Hazel, Hieab, Joyce, Jorge, Jory, Kèren, Lotte, Mariëlle, Marileen, Michiel, Renée, Rens, Renske, Saloua, Saira, Tom, en Vincent K. en Vincent V. dank ik voor de goede werksfeer en gezelligheid. Hazel en Saloua, mijn kamergenootjes in de nieuwbouw, jullie humor is van grote klasse. Ik zou graag nog eens met jullie willen grunten door de telefoon. Eline, Hieab en Vincent V., wat hadden we samen toch interessante komische gesprekken in kamer 21-81, vaak resulterend in de bekende bulderende lach van Vincent V. en de hoogpiekende giegelende lach van Eline. Dankzij het hoge aantal decibels kon ik toen ik verhuisde naar een paar deuren verderop er gelukkig nog altijd van meegenieten. Later toen Eline gepromoveerd was kreeg ze een waardige vervanger in de persoon van Renée, die niet alleen net zo lief was, maar ook hetzelfde aantal decibels haalde met haar lach. In mijn nieuwe kamer zaten Mariëlle, Jory, soms Tom, en later Hieab en Elizabeth, waar ook de nodige grapjes werden gemaakt en natuurlijk ook gewerkt werd.

Ook de beeldverwerkers met wie ik veel heb samengewerkt wil ik bedanken: Dirk, Esther, Eugene, Fedde, Hakim, Henri, Marcel, Marius, Marleen, Renske, Stefan en Wiro. Ik heb veel van jullie geleerd.

Bedankt ook mijn gewaardeerde collega's van de afdeling Epidemiologie en Radiologie: Abbas, Ayesha, Andres, Annemarie, Bart, Bas, Bob, Bouwe, Carla, Carolina M.G., Carolina M.O., Caroline, Charlotte, Daan, Edith, Eline, Farzin, Gabriëlle, Germaine, Heidi, Henriëtte, Janine, Karen, Karin, Laura, Lennart, Linda, Ling, Lintje, Lisette, Maarten, Mariana, Marijn, Mark, Marlies, Maryam, Matthijs, Milad, Monika, Najaf, Nathalie, Nese, Nikkie, Paul, Quirijn, Rachel, Raymond, Rebecca, Rikje, Rob, Rozanne, Sanaz, Sandra, Sjel, Suman, Sven, Symen, Tessa, Teun, Toke, Vera, Virginie, en Wishal.

Lieve familie, jullie zijn van onschatbare waarde. Lieve papa en mama, mijn dank is niet in woorden uit te drukken. Jullie staan altijd klaar en weten altijd raad. Lieve Mark, bedankt dat je zo een fijne broer bent. Lieve opa en oma's, bedankt voor jullie warmte en gezelligheid.
Lieve vrienden, dear friends, bedankt voor jullie afleiding, ondersteuning en gezelligheid. Beste David, Hazem, Ibrahim, Sander, Vincent K. en Wing, jullie in het bijzonder bedankt. Beste Hazem, ik ben blij dat jij mijn paranimf wilt zijn. Jij hebt me ooit op het idee gebracht om te gaan promoveren. We hebben vaak grappige gesprekken over wetenschap, filosofie en andere zaken. Ik hoop dat we dat nog vaak kunnen doen. Beste Vincent V. ik ben blij dat ook jij mijn paranimf wilt te zijn. Ik vond het erg gezellig met jou op de kamer en heb goede herinningen aan onze Colombiaanse "dinners".

Lieve Ingrid, ik ben heel gelukkig met jou, Alejandro en ons kindje in spé. Ik hoop dat we nog lang van elkaar kunnen genieten. Bedankt voor je begrip en ondersteuning in de afgelopen intensieve periode.

PHD PORTFOLIO

1. PhD training		
	Year	**Workload (Hours/ECTS)**
General courses		
- MSc Clinical Epidemiology	2009	42.0
- Biomedical English Writing and Communication	2011	4.0
Specific courses (e.g. Research school, Medical Training)		
- Peripheral and Intracranial Obstructive Vascular Disease (Coeur, Erasmus MC)	2009	1.5
- Cardiovascular Imaging and diagnostics (Coeur, Erasmus MC)	2010	1.5
- SNPs and Complex diseases (NIHES /Molmed, Erasmus MC)	2010	1.4
- Basic course on R (NIHES /Molmed, Erasmus MC)	2010	0.6
- Linux for scientists (NIHES/Molmed, Erasmus MC)	2011	0.6
- Practical Course Running GWAS (NIHES/Molmed, Erasmus MC)	2011	0.6
(Inter)national conferences and presentations		
- *Automated classification of periventricular and subcortical white matter lesions on MRI*. Alzheimer's Association International Conference on Alzheimer's Disease, Vienna (Austria). Poster presentation.	2009	1.0
- *Identifying causes and preclinical markers of dementia and stroke*. Netherlands Consortium for Healthy Ageing, Unilever, Rotterdam (Netherlands). Poster presentation.	2010	1.0
- *Are SNPs associated with Alzheimer's disease also associated with cognition and structural brain changes in a relatively young population?* Alzheimer's Association International Conference on Alzheimer's Disease, Paris (France). Poster presentation.	2011	1.0
- *Is a risk score based on Alzheimer's disease SNPs associated with cognition in the general population?* VAS-COG conference, Lille (France). Poster presentation.	2011	1.0
- *The relation of uric acid with vascular brain disease and cognition: The Rotterdam Scan Study.* VAS-COG conference, Lille (France). Poster presentation.	2011	1.0
- CHARGE consortium meeting, Los Angeles (US).	2011	1.0
- *The influence of blood pressure SNPs on the cerebral vasculature.* CHARGE-meeting, Reykjavik (Iceland). Poster presentation.	2012	1.0
- *Genetic studies on preclinical markers of dementia.* Netherlands Consortium on Healthy Ageing, Leiden University Medical Center, Leiden (Netherlands). Oral presenation.	2012	1.0
- European Congress of Radiology, Vienna (Austria).	2013	1.0
- *High blood pressure and cerebral white matter lesion progression in the general population.* European Congress of Radiology, Vienna (Austria). Oral presentation.	2013	1.0
- *The relation of uric acid with brain atrophy and cognition: the Rotterdam scan study.* European Congress of Radiology, Vienna (Austria). Oral presentation.	2013	1.0
- *Subtle changes in normal appearing white matter precede development of white matter lesions.* European Congress of Radiology, Vienna (Austria). Oral presentation.	2013	1.0
- Netherlands Consortium for Healthy Ageing Congress, The Hague (Netherlands).	2013	1.0
- *Second wave meta-GWAS on cerebral white matter lesions identifies new locus.* CHARGE-meeting (Spring), Rotterdam (the Netherlands). Poster presentation.	2013	1.0

2. Teaching		
	Year	**Workload (Hours/ECTS)**
Teaching assistant for course 'Principles of Research in Medicine' at the Erasmus Summer Programme	2010/2011	2.0
Supervising practicals and excursions, Tutoring - Supervising practicals on statistics - Supervising practicals on epidemiology	 2010 2010	 1.0 1.0
Supervising Master's theses - Supervised Jorge Cárdenas Roldán: Kidney function and cognition	 2009/2010	 6.0
Other - Exam trainer in epidemiology and statistics for medical students	 2010/2011	 2.0

PUBLICATIONS

Adams HH, **Verhaaren BF**, Vrooman HA, Uitterlinden AG, Hofman A, Van Duijn CM, Van der Lugt A, Niessen WJ, Vernooij MW, Ikram MA. TMEM106B influences volume of left-side temporal lobe and interhemispheric structures in the general population. Biol Psychiatry, in press.

Malik R, Bevan S, Nalls MA, Holliday EG, Devan WJ, Cheng YC, Ibrahim-Verbaas CA, **Verhaaren BF**, Bis JC, Joon AY, de Stefano AL, Fornage M, Psaty BM, Ikram MA, Launer LJ, van Duijn CM, Sharma P, Mitchell BD, Rosand J, Meschia JF, Levi C, Rothwell PM, Sudlow C, Markus HS, Seshadri S, Dichgans M; Wellcome Trust Case Control Consortium 2. Multilocus genetic risk score associates with ischemic stroke in case-control and prospective cohort studies. *Stroke*. 2014 Feb;45(2):394-402.

Adams HH, Cavalieri M, **Verhaaren BF**, Bos D, van der Lugt A, Enzinger C, Vernooij MW, Schmidt R, Ikram MA. Rating method for dilated Virchow-Robin spaces on magnetic resonance imaging. Stroke. 2013 Jun;44(6):1732-5.

Verhaaren BF, Vernooij MW, Dehghan A, Vrooman HA, de Boer R, Hofman A, Witteman JC, Niessen WJ, Breteler MM, van der Lugt A, Ikram MA. The relation of uric acid to brain atrophy and cognition: the Rotterdam Scan Study. Neuroepidemiology. 2013;41(1):29-34.

Verhaaren BF, Vernooij MW, de Boer R, Hofman A, Niessen WJ, van der Lugt A, Ikram MA. High blood pressure and cerebral white matter lesion progression in the general population. Hypertension. 2013 Jun;61(6):1354-9.

de Groot M*, **Verhaaren BF***, de Boer R, Klein S, Hofman A, van der Lugt A, Ikram MA, Niessen WJ, Vernooij MW. Changes in normal-appearing white matter precede development of white matter lesions. Stroke. 2013 Apr;44(4):1037-42.

Bos D, Ikram MA, Isaacs A, **Verhaaren BF**, Hofman A, van Duijn CM, Witteman JC, van der Lugt A, Vernooij MW. Genetic loci for coronary calcification and serum lipids relate to aortic and carotid calcification. Circ Cardiovasc Genet. 2013 Feb;6(1):47-53.

Holliday EG, Maguire JM, Evans TJ, Koblar SA, Jannes J, Sturm JW, Hankey GJ, Baker R, Golledge J, Parsons MW, Malik R, McEvoy M, Biros E, Lewis MD, Lincz LF, Peel R, Oldmeadow C, Smith W, Moscato P, Barlera S, Bevan S, Bis JC, Boerwinkle E, Boncoraglio GB, Brott TG, Brown RD Jr, Cheng YC, Cole JW, Cotlarciuc I, Devan WJ, Fornage M, Furie KL, Grétarsdóttir S, Gschwendtner A, Ikram MA, Longstreth WT Jr, Meschia JF, Mitchell BD, Mosley TH, Nalls MA, Parati EA, Psaty BM, Sharma P, Stefansson K, Thorleifsson G, Thorsteinsdottir U, Traylor M, **Verhaaren BF**, Wiggins KL, Worrall BB, Sudlow C, Rothwell PM, Farrall M, Dichgans M, Rosand J, Markus HS, Scott RJ, Levi C, Attia J; Australian Stroke Genetics Collaborative; International Stroke Genetics Consortium; Wellcome Trust Case Control Consortium 2. Common variants at 6p21.1 are associated with large artery atherosclerotic stroke. Nat Genet. 2012 Oct;44(10):1147-51.

Verhaaren BF, Vernooij MW, Koudstaal PJ, Uitterlinden AG, van Duijn CM, Hofman A, Breteler MM, Ikram MA. Alzheimer's disease genes and cognition in the nondemented general population. Biol Psychiatry. 2013 Mar 1;73(5):429-34.

Schrijvers EM, **Verhaaren BF**, Koudstaal PJ, Hofman A, Ikram MA, Breteler MM. Is dementia incidence declining?: Trends in dementia incidence since 1990 in the Rotterdam Study. Neurology. 2012 May 8;78(19):1456-63.

Bos D, Vernooij MW, Elias-Smale SE, **Verhaaren BF**, Vrooman HA, Hofman A, Niessen WJ, Witteman JC, van der Lugt A, Ikram MA. Atherosclerotic calcification relates to cognitive function and to brain changes on magnetic resonance imaging. Alzheimers Dement. 2012 Oct;8(5 Suppl):S104-11.

Bis JC, DeCarli C, Smith AV, van der Lijn F, Crivello F, Fornage M, Debette S, Shulman JM, Schmidt H, Srikanth V, Schuur M, Yu L, Choi SH, Sigurdsson S, **Verhaaren BF**, DeStefano AL, Lambert JC, Jack CR Jr, Struchalin M, Stankovich J, Ibrahim-Verbaas CA, Fleischman D, Zijdenbos A, den Heijer T, Mazoyer B, Coker LH, Enzinger C, Danoy P, Amin N, Arfanakis K, van Buchem MA, de Bruijn RF, Beiser A, Dufouil C, Huang J, Cavalieri M, Thomson R, Niessen WJ, Chibnik LB, Gislason GK, Hofman A, Pikula A, Amouyel P, Freeman KB, Phan TG, Oostra BA, Stein JL, Medland SE, Vasquez AA, Hibar DP, Wright MJ, Franke B, Martin NG, Thompson PM; Enhancing Neuro Imaging Genetics through Meta-Analysis Consortium, Nalls MA, Uitterlinden AG, Au R, Elbaz A, Beare RJ, van Swieten JC, Lopez OL, Harris TB, Chouraki V, Breteler MM, De Jager PL, Becker JT, Vernooij MW, Knopman D, Fazekas F, Wolf PA, van der Lugt A, Gudnason V, Longstreth WT Jr, Brown MA, Bennett DA, van Duijn CM, Mosley TH, Schmidt R, Tzourio C, Launer LJ, Ikram MA, Seshadri S; Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium. Common variants at 12q14 and 12q24 are associated with hippocampal volume. Nat Genet. 2012 Apr 15;44(5):545-51.

Taal HR, Verwoert GC, Demirkan A, Janssens AC, Rice K, Ehret G, Smith AV, **Verhaaren BF**, Witteman JC, Hofman A, Vernooij MW, Uitterlinden AG, Rivadeneira F, Ikram MA, Levy D, van der Heijden AJ; Cohort for Heart and Aging Research in Genome Epidemiology and Early Genetics and Lifecourse Epidemiology consortia, Jaddoe VW, van Duijn CM. Genome-wide profiling of blood pressure in adults and children. Hypertension. 2012 Feb;59(2):241-7.

van der Lijn F, **Verhaaren BF**, Ikram MA, Klein S, de Bruijne M, Vrooman HA, Vernooij MW, Hammers A, Rueckert D, van der Lugt A, Breteler MM, Niessen WJ. Automated measurement of local white matter lesion volume. Neuroimage. 2012 Feb 15;59(4):3901-8.

Poels MM*, **Verhaaren BF***, Hoogendam YY*, Ikram MA*, Bos D*, Vernooij MW. *De Rotterdam Scan Study:* een grootschalig MRI-onderzoek naar hersenziekten Neuropraxis, Volume 16, Number 1, 14-23, 2012.

Verhaaren BF, de Boer R, Vernooij MW, Rivadeneira F, Uitterlinden AG, Hofman A, Krestin GP, van der Lugt A, Niessen WJ, Breteler MM, Ikram MA. Replication study of chr17q25 with cerebral white matter lesion volume. Stroke. 2011 Nov;42(11):3297-9.

Abdul-Hussien H, Hanemaaijer R, Kleemann R, **Verhaaren BF**, van Bockel JH, Lindeman JH. The pathophysiology of abdominal aortic aneurysm growth: corresponding and discordant inflammatory and proteolytic processes in abdominal aortic and popliteal artery aneurysms. J Vasc Surg. 2010 Jun;51(6):1479-87.

*Contributed equally.

ABOUT THE AUTHOR

Benjamin F.J. Verhaaren was born in Great Yarmouth, United Kingdom, on August 24th 1984. After graduating from the 'Alfrink College' in Zoetermeer (2002) he started medical school at Leiden University, Leiden. During his medical training Benjamin took elective clerkships in Orthopedics at the Dr. Ram Manohar Lohia Hospital in Delhi, India (2005), in Cardiovascular surgery at the Universitätsklinikum Freiburg, Freiburg, Germany (2006), and performed research at the department of Vascular Surgery at the Leiden University Medical Center, Leiden, the Netherlands (2006/2007). He obtained his master degree in Medicine cum laude (2009) and his medical degree (2010) cum laude. In 2009 he obtained a master degree in Clinical Epidemiology from the Netherlands Institute for Health Sciences, Rotterdam. In 2010 he started the work described in this thesis at the department of Epidemiology (head: Prof. Dr. A. Hofman) and Radiology (head: Prof. Dr. G.P. Krestin) at the Erasmus MC under supervision of Prof. Dr. A. Hofman, Prof. Dr. A. van der Lugt, Dr. M.A. Ikram, and Dr. M.W. Vernooij. His paper with his colleague Marius de Groot entitled “Changes in normal-appearing white matter precede development of white matter lesions” was chosen as the 1st prize winner of the *2013 Stroke Progress and Innovation Award* by the scientific journal ‘Stroke’. As of September 2013, Benjamin started his residency in Radiology at the Erasmus MC, Rotterdam in the Netherlands.