Lower microstructural integrity of brain white matter is related to higher mortality

Lotte G.M*. Cremers, Sanaz Sedaghat*, Marius de Groot, Albert Hofman, Aad van der Lugt, Wiro J. Niessen, Oscar H. Franco, Abbas Dehghan, M. Arfan Ikram, Meike W. Vernooij

Neurology 2015

**Denotes equal contribution*

ABSTRACT

Objective To investigate the association of cerebral white matter microstructural integrity with mortality.

Methods We included 4294 individuals, free from stroke and dementia (mean age 63.6 years, 44% male) from the population-based Rotterdam Study (2006-2011). Diffusion-MRI was used to assess the microstructural integrity of the white matter, both globally and for specific white matter tracts. Fractional anisotropy (FA) and mean diffusivity (MD) were the parameters used to quantify white matter integrity. All-cause mortality and cause-specific mortality was recorded with a median follow-up time of 5.4 year and 4.6 years, respectively. Cox regression models, adjusted for age, sex, *APOE*-ε4 allele carriership, cardiovascular risk factors and macrostructural MRI changes, were used to estimate hazard ratios.

Results During the follow-up time 216 (5.0%) participants died from all causes, 31 (0.7%) from cardiovascular causes and 102 (2.4%) individuals died of non-cardiovascular causes. Each standard deviation (SD) decrease in FA and each SD increase in MD was associated with a 1.37 fold (95%CI: 1.20, 1.57) and a 1.49 fold (95%CI: 1.28, 1.75) higher hazard of all-cause mortality, respectively. The associations were more prominent with cardiovascular mortality rather than non-cardiovascular mortality. In tract-specific analyses, we observed that association tracts were more prominently related to mortality.

Conclusions Our findings suggest that impairments in cerebral white matter, even at early stages, are not limited to adverse brain outcomes and they are related to mortality especially from cardiovascular causes.

INTRODUCTION

Brain white matter plays a major role in brain functioning e.g. in cognitive function and motor function.[1,2] One of the manifestations of white matter damage is the emergence of white matter hyperintensities, a common finding on MRI.[3] Several studies reported a link between presence of white matter hyperintensities and adverse health outcomes, as well as shorter survival.[4-6] However, it seems that white matter hyperintensities could constitute only the "tip of the iceberg" of white matter pathology, and changes in the microstructure of white matter develop long before appearance of white matter hyperintensities on MRI.[7] Diffusion-MRI is a sensitive MRI technique, to quantify subtle changes in the white matter microstructure. Fractional anisotropy (FA) and mean diffusivity (MD) are commonly used diffusion-MRI parameters. Generally, lower FA and higher MD are associated with poorer white matter microstructural integrity. Given the prominent role of white matter hyperintensities not only in development and progression of brain disorders as dementia and stroke[8,9] but also in poor survival[10], it is of great importance to study early changes in white matter before white matter hyperintensities form.[11] Therapeutic approaches and life style changes in early phase might help to prevent further progression of impairments in white matter microstructural integrity.[11,12]
We hypothesized that early stage white matter pathologies are associated with shorter survival. We investigated the association of cerebral microstructural integrity with mortality. Previous studies showed that different white matter tracts have differences in vulnerability to degeneration.[13,14] Therefore, we assessed whether regional differences in white matter microstructural integrity had differential effects on mortality.

METHODS

Population

The present study is embedded within the framework of the Rotterdam Study, an ongoing prospective population-based study, among inhabitants of Ommoord, a district of Rotterdam in the Netherlands. The design of the Rotterdam Study has been described previously.[15,16] Since 2005, brain MRI is implemented into the study protocol of the Rotterdam Study. Between 2006 and 2011, out of 5430 non-demented eligible participates, 4841 persons underwent a structural and diffusion-MRI of the brain. We excluded 53 scans due to incomplete acquisitions, 112 scans due to artifacts hampering automated processing, 135 scans due to failed tissue segmentation, and 160 scans due to presence of cortical infarcts (MRI-defined). We additionally excluded participants with history of clinical stroke (n=87). Persons with cortical infarcts and stroke were excluded from all analyses because tissue loss and the gliosis surrounding infarcts may influence image registration resulting in unreliable WMH segmentation volumes. This

resulted in 4294 participants for both global and tract-specific analyses (age range: 45.7-100.0).

Standard Protocol Approvals, Registrations, and Patient Consents

The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.[15]

MRI acquisition, processing and inspection

Brain MRI scanning was performed on a 1.5T MRI scanner (GE Signa Excite). Scan protocol and sequence details are described extensively elsewhere.[16] For the diffusion scan, a single shot, diffusion weighted spin echo echo-planar imaging sequence was performed (maximum b-value was 1000 s/mm^2 in 25 non-collinear directions, three volumes were acquired without diffusion weighting (b-value = 0 s/mm^2)).[16] The T1-weighted, proton, density-weighted and the fluid-attenuated inversion recovery scans were used for automated segmentation of grey matter, white matter, white matter hyperintensities (WMH), which was extended with a post-processing WMH segmentation approach.[17-19] All segmentation results were visually inspected and, if needed manually corrected. Supratentorial intracranial volume (ICV) was estimated by summing grey and white matter, and CSF volumes.[17] Cortical infarcts were rated on structural sequences, and in case of involvement of cortical grey matter, they were classified as cortical infarcts. Lacunes were defined as focal hyperintensities (size ≥3 and < 15 mm) with the signal intensity of CSF on all sequences, and when located supratentorially with a hyperintense rim on fluid-attenuated inversion recovery (FLAIR) sequence. To differentiate lacunar infarcts from dilated perivascular spaces, symmetry of the lesions, sharp demarcation and absence of a hyperintense rim on the FLAIR sequence supported presence of a dilated perivascular space.[20] Cerebral microbleeds were rated on a three-dimensional T2*-weighted gradient-recalled echo MRI scan as focal areas of very low signal intensity.[16]

Diffusion-MRI processing and tractography

All diffusion data were pre-processed using a standardized pipeline (including correction for motion and eddy currents), obtaining global mean FA and MD in the normal-appearing white matter (voxels with WMH were excluded from the global analysis). White matter tracts were segmented using a probabilistic diffusion tractography approach described previously.[21] In tract-specific analyses voxels with WMH were not excluded. We segmented 14 different white matter tracts (11 tracts were present in the left and right hemispheres), and obtained participant specific median scores for FA

and MD inside each white matter tracts, with subsequent combination of left and right measures. The resulting tract-specific means were standardized.[21] Tracts were categorized based on anatomy, into brainstem tracts, projection tracts, association tracts, limbic system tracts, and callosal tracts.[22] The tract segmentations were used to obtain tract-specific white matter volumes, and by combining tissue and tract segmentations we obtained tract-specific WMH volumes.

Mortality

Deaths (in and out-hospital) were reported on a weekly basis through the automatic linkage with General Practitioner (GP) files. In addition, central registry of the municipality in Rotterdam was checked bimonthly for information on vital status. For participants moved outside the research area, the GPs were the primary source of information, complemented by the municipality records in the place of residence. This also includes people in nursing homes. For cause-specific mortality, research physicians reviewed all available information (from general practitioner and hospital records) and coded the events according to the International Classification of Diseases, 10th edition (ICD-10). Death due to cardiovascular mortality was classified as ICD-10 codes I00-I99 and death due to other reasons was recorded as non-cardiovascular mortality. A consensus panel, led by a physician with expertise in cardiovascular disease, decided the final cause of death according to ICD-10 codes using standardized definitions. Follow-up was completed until July 4, 2014 and January 1, 2013 for total mortality and cause-specific mortality, respectively.[23]

Cardiovascular risk factors

After a resting period of five minutes, blood pressure was measured twice in a single visit using a random-zero sphygmomanometer. Total and high density lipoprotein (HDL) cholesterol levels were determined using an automated enzymatic method. Information on smoking and antihypertensive and lipid lowering medication was based on home interviews. Smoking was categorized in never, former and current smoking. Cardiovascular disease was considered as a history of myocardial infarction, or coronary revascularization procedures.[23] Diabetes mellitus was defined by use of blood glucose lowering medication or a fasting serum glucose level equal to or greater than 7.0 mmol/l.[7] Apolipoprotein E (*APOE*) ε4 allele carriership was assessed on coded genomic DNA samples.

Statistical analysis

Due to a skewed distribution of volume of WMH, we natural-log transformed WMH volumes. Associations of white matter microstructural integrity (global and tract-specific) with all-cause mortality were evaluated using Cox proportional hazard models.

To take into account the competing risk, for cardiovascular and non-cardiovascular mortality we used a competing risk approach (R package "riskRegression").
We performed the analyses in four models. In the first model we adjusted for age, and sex. In the second model, analyses were adjusted additionally for cardiovascular risk factors including systolic blood pressure, diastolic blood pressure, antihypertensive medication, total and HDL cholesterol, lipid-lowering medication, smoking, history of coronary heart disease, diabetes mellitus, body mass index, and *APOE*-ε4 allele carriership. In the third model, we adjusted for age, sex, macrostructural white matter changes ((tract-specific) white matter volume, and log transformed (tract-specific) volume of white matter hyperintensities), intracranial volume, presence of microbleeds, and presence of lacunar infarcts. In both the global and tract-specific analysis we adjusted for WMH volume to control for possible partial volume effects and to single out real microstructural changes.[7] Model four was adjusted for all covariates from first, second and third models. Tract-specific analyses were performed with model three.[21] Since the cerebellum could not always be fully incorporated in the diffusion scan leading to a varying coverage of the brainstem tracts (mainly medial lemniscus), we additionally controlled for this factor in the tract-specific analyses of the medial lemniscus.[22] Linearity and proportionality assumptions were met for all analyses. We performed multiple imputation for missing data in the covariates (< 12% for all covariates), using a Markov Chain Monte Carlo method and we used the imputed data for all the analyses.
We inspected the mortality rates per 1000 person-years in tertiles of FA and MD, and to take into account the differences in age and sex, we made tertiles of FA and MD based on the residuals of FA and MD regressed against age and sex. We additionally used the Kaplan-Meier method to estimate cumulative mortality curves of all-cause mortality associated with the tertiles of FA and MD. To investigate whether damage to the microstructural integrity of white matter is associated with mortality due to causes other than neurological diseases, we performed a series of sensitivity analyses. First, participants with interim dementia or clinically reported stroke were censored to rule out the influence of new cases of dementia and stroke during follow-up on the association of FA and MD with all-cause mortality.[24] Second, we repeated the association of FA and MD with cardiovascular mortality after excluding deaths due to stroke.
For the tract-specific analysis we used Šidák correction to correct for multiple comparisons, after estimating the number of independent tests,[25] resulting in $p < 0.0037$ as the significance threshold for an alpha value of 0.05. All analyses were carried out using SPSS 20.0.2 for Windows or R version 2.15.0.

RESULTS

Baseline characteristics of the participants are presented in **Table 1**. Comparing eligible and non-eligible participants, we observed that excluded participants were older and had higher loads of cardiovascular risk factors **(Table e-1)**.

Table 1. Baseline characteristics

Baseline characteristics	**N= 4294**
Age, years	63.6 (11.0)
Age of death, years	72.6 (11.9)
Men	1906 (44.4)
Systolic blood pressure, mmHg	139.0 (21.5)
Diastolic blood pressure, mmHg	83.1 (10.8)
Antihypertensive medication	1463 (34.1)
Total cholesterol, mmol/l	5.5 (1.0)
HDL cholesterol, mmol/l	1.4 (0.4)
Lipid-lowering medication	1014 (23.6)
Smoking	
Current	878 (20.4)
Former	2060 (48.0)
History of coronary heart disease	256 (6.0)
Diabetes mellitus	393 (9.2)
Body mass index, kg/m^2	27.4 (4.1)
*APOE-ε*4 allele carrier	1140 (28.4)
White matter volume, mL	403.3 (60.8)
Volume of white matter hyperintensities, mL	4.4 (2.4,8.8)
Intracranial volume, mL	1340.0 (132.9)
Cerebral microbleeds	779 (18.1)
Lacunar infarcts	93 (2.2)
Fractional anisotropy	0.34 (0.01)
Mean diffusivity, 10^{-3} mm^2/s	0.74 (0.03)

Categorical variables are presented as numbers (percentages), continuous variables as means (standard deviations) and volume of white matter hyperintensities is presented as median (interquartile range).
The following variables had missing data: blood pressure (n=90), blood pressure lowering medication (n=78), smoking (n=69), lipid-lowering medication (n= 48), HDL: high density lipoprotein cholesterol (n=520), total cholesterol (n= 518), body mass index (n=82), diabetes (n=125), history of coronary heart disease (n=44), *APOE-ε*4 allele carrier (n=273).
Abbreviation: *APOE*: Apolipoprotein E.Comparing eligible and non-eligible participants, we observed that excluded participants were older and had higher loads of cardiovascular risk factors **(Table e-1)**.

During the median [interquartile range] follow-up time of 5.4 [3.6-5.9] years, 216 (5%) participants died. For cause-specific mortality, during the median [interquartile range] follow-up time of 4.6 [3.3-5.7] years, 31 (0.7%) individuals died of cardiovascular causes

and 102 (2.4%) participants died from non-cardiovascular causes. All-cause mortality rates in participants with low, middle and high tertiles of FA and MD are shown in **Figure 1**.

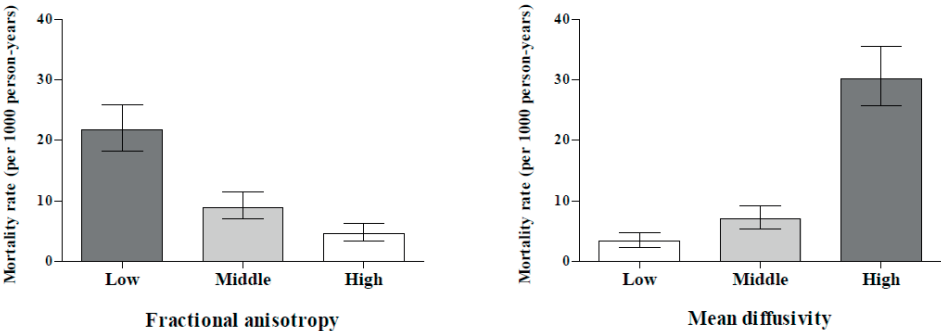


Figure 1

In model I, adjusted for age and sex, decrease in FA and increase in MD was associated with higher hazard of all-cause mortality. Adjustments for cardiovascular risk factors, macrostructural MRI changes and presence of lacunar infarcts and microbleeds attenuated the associations minimally (**Table 2)**.

Table 2. The association between white matter microstructural integrity and mortality

	All-cause mortality	
	Hazard ratio* (95% CI) N=4294 (216)	**p value**
Fractional anisotropy		
Model I	1.37 (1.20, 1.57)	<0.001
Model II	1.31 (1.15, 1.49)	<0.001
Model III	1.27 (1.10, 1.48)	0.001
Model IV	1.24 (1.10, 1.43)	0.004
Mean diffusivity		
Model I	1.49 (1.28, 1.75)	<0.001
Model II	1.39 (1.19, 1.63)	<0.001
Model III	1.39 (1.17, 1.66)	<0.001
Model IV	1.32 (1.11, 1.56)	0.002

*Hazard ratios and 95% CI are calculated per standard deviation decrease in fractional anisotropy and per standard deviation increase in mean diffusivity.

Model I: Adjusted for age, and sex.

Model II: Model I + systolic blood pressure, diastolic blood pressure, antihypertensive medication, smoking, total cholesterol, high density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, history of coronary heart disease, *APOE-ε*4 allele carrier, and body mass index.

Model III: Model I + intracranial volume, white matter volume, logarithm of white matter lesion volume, presence of microbleeds, and presence of lacunar infarcts.

Model IV: Model I+ II + III

Abbreviation: CI: confidence interval, *APOE*: Apolipoprotein E.

Each SD decrease in FA was associated with 1.99-fold increased hazard of cardiovascular mortality (95% CI: 1.44, 2.77), in the age and sex adjusted model. Further adjustments did not change our findings. There was no association between FA and non-cardiovascular mortality (all p>0.05). Similarly, each SD higher MD was associated with 1.84-fold increased hazard of cardiovascular mortality (95%CI: 1.26, 2.68). After adjustments for cardiovascular risk factors and macrostructural MRI changes and presence of lacunar infarcts and microbleeds, the associations attenuated and were no longer significant (p= 0.207). Higher MD was associated with increased hazard of non-cardiovascular mortality (HR: 1.31, 95%CI: 1.04, 1.65), but the association was no longer significant after further adjustments (**Table 3**).

Table 3. The association of white matter microstructural integrity and cardiovascular and non-cardiovascular mortality

	Cardiovascular mortality		**Non-cardiovascular mortality**	
	Hazard ratio* (95% CI) N=4294 (31)	**p value**	**Hazard ratio* (95% CI) N=4294 (102)**	**p value**
Fractional anisotropy				
Model I	1.99 (1.44, 2.77)	<0.001	1.19 (0.98, 1.44)	0.079
Model II	1.81 (1.28, 2.57)	0.001	1.13 (0.93, 1.38)	0.206
Model III	1.62 (1.12, 2.34)	0.010	1.07 (0.87, 1.33)	0.513
Model IV	1.53 (1.04, 2.24)	0.029	1.04 (0.84, 1.28)	0.735
Mean diffusivity				
Model I	1.84 (1.26, 2.68)	0.001	1.31 (1.04, 1.65)	0.021
Model II	1.66 (1.10, 2.49)	0.015	1.22 (0.97, 1.54)	0.085
Model III	1.48 (0.96, 2.30)	0.077	1.12 (0.86, 1.46)	0.381
Model IV	1.34 (0.85, 2.13)	0.207	1.07 (0.82, 1.38)	0.620

*Hazard ratios and 95% CI are calculated per standard deviation decrease in fractional anisotropy and per standard deviation increase in mean diffusivity.

Model I: Adjusted for age, and sex.

Model II: Model I + systolic blood pressure, diastolic blood pressure, antihypertensive medication, smoking, total cholesterol, high density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, history of coronary heart disease, *APOE-ε4* allele carrier, and body mass index.

Model III: Model I + intracranial volume, white matter volume, logarithm of white matter lesion volume, presence
of microbleeds, and presence of lacunar infarcts.

Model IV: Model I+ II + III

Abbreviation: CI: confidence interval; *APOE:* Apolipoprotein E.

Kaplan-Meier survival curves showed that individuals with low FA and high MD had the highest cumulative mortality rate (**Figure e-1**).

In the tract-specific analyses, we observed that for all tracts, MD was more prominently related to mortality than FA. For FA, after correcting for multiple testing, lower microstructural integrity in the different white matter tracts was not associated with mortality. For MD, the association between lower tract-specific white matter microstructural integrity and mortality seemed to be present throughout the brain; however, it was more prominent in the association tracts (**Table 4** and **Figure e-2**).

Excluding participants with interim dementia or stroke did not change our observations (**Table e-2**). Similarly, excluding stroke death did not change the association of FA and MD with cardiovascular mortality (**Table e-3**).

Table 4. Associations of tract-specific white matter microstructural integrity and mortality

	Fractional Anisotropy	**Mean diffusivity**
	Hazard ratio* (95% CI)	Hazard ratio* (95% CI)
Tracts in brainstem		
Middle cerebellar peduncle	1.04 (1.27, 0.86)	1.08 (0.95, 1.22)
Medial lemniscus [a]	1.03 (1.22, 0.87)	**1.21 (1.06, 1.37)***
Projection tracts		
Corticospinal tract	1.06 (1.22, 0.93)	**1.29 (1.11, 1.49)***
Anterior thalamic radiation	1.05 (1.23, 0.89)	**1.17 (1.00, 1.37)**
Superior thalamic radiation	1.06 (1.22, 0.93)	**1.21 (1.05, 1.38)**
Posterior thalamic radiation	1.19 (1.43, 1.00)	1.10 (0.95, 1.27)
Association tracts		
Superior longitudinal fasciculus	**1.20 (1.41, 1.03)**	**1.28 (1.13, 1.46)***
Inferior longitudinal fasciculus	1.09 (1.30, 0.92)	**1.28 (1.09, 1.49)***
Inferior fronto-occipital fasciculus	**1.25 (1.49, 1.04)**	1.24 (1.05, 1.45)
Uncinate fasciculus	**1.27 (1.49, 1.06)**	**1.35 (1.15, 1.57)***
Limbic system tracts		
Cingulate gyrus part of cingulum	1.09 (1.28, 0.93)	**1.27 (1.10, 1.47)***
Parahippocampal part of cingulum	1.14 (1.30, 0.97)	1.00 (0.91, 1.10)
Callosal tracts		
Forceps major	**1.28 (1.52, 1.06)**	1.08 (0.96, 1.23)
Forceps minor	**1.23 (1.49, 1.02)**	**1.24 (1.06, 1.45)**

*Hazard ratios and 95% CI, calculated per standard deviation decrease in fractional anisotropy and increase in mean diffusivity, adjusted for age, sex, intracranial volume, white matter volume, volume of white matter hyperintensities, presence of microbleeds, and presence of lacunar infarcts.

Results in bold are significant at $p<0.05$.

Results in bold and * were significant after correction for multiple testing ($p< 3.7 \times 10^{-3}$).

Abbreviation: CI: confidence interval.

DISCUSSION

We showed that among community-dwelling individuals, lower microstructural integrity of cerebral white matter is related to an increased mortality, independent of cardiovascular risk factors, macrostructural MRI changes (white matter volume and the log transformed volume of white matter hyperintensities), and presence of lacunar infarcts and microbleeds. The association was more prominent with cardiovascular mortality rather than non-cardiovascular mortality. Furthermore, we found that lower white matter microstructure in the association tracts most strongly related to mortality.
White matter is a key component of the brain consisting of glial cells and myelinated axons that transmit signals mostly between various regions of the brain and spinal cord.[2] White matter accounts for nearly half of the brain volume and its microstructural integrity is crucial for normal brain function.[7] White matter damage represented as white matter hyperintensities and white matter atrophy is reported to be related to a higher risk of mortality.[4,10,26] The association between more subtle, microstructural changes in white matter can provide a better insight into cerebral white matter pathology, but this has not yet been investigated in a population-based setting. Virtanen et al., in pre-operative patients with peripheral arterial disease, showed that microstructural changes in white matter are linked to a worse cardiorespiratory and metabolic profile and an increased risk of mortality.[27] In this study we showed that this finding can be extended to the general population free of dementia and stroke.
We observed that poorer white matter microstructural integrity was associated with higher all-cause, cardiovascular, and non-cardiovascular mortality, but most prominently with cardiovascular mortality. A possible explanation for this observation could be that white matter is more prone to vascular insults.[28,29] Shared cardiovascular risk factors such as hypertension and diabetes might play a role in this association. Adjusting for cardiovascular risk factors attenuated the associations; but the findings persisted. This might indicate that at least part of the association is driven by cardiovascular risk factors that could not be measured, or represent the very initial phase of atherosclerotic processes. Rather than cardiovascular risk factors, one of the important pathologies that would influence white matter integrity is neurodegenerative diseases such as Alzheimer's disease. Although we excluded the dementia cases from the analyses, we cannot exclude the possibility that impairment in the white matter integrity could be related to (preclinical) neurodegenerative processes, in particular in the association tracts.
Available literature suggests that damage to cerebral white matter is not only associated with deterioration in cerebral functions such as cognition, sensory or motor function but also with disturbances in regulation of endocrine and autonomic nervous systems throughout the body.[30] Disturbances in white matter microstructural integrity

may therefore influence a variety of functions throughout the body that may result in a higher risk of mortality, also due to causes other than neurological diseases.[6,30] This is in line with our observation that adjusting for macrostructural-MRI changes (white matter volume, and log transformed volume of white matter hyperintensities) and excluding neurological diseases (interim stroke and dementia) did not change our findings. Moreover, the association between white matter microstructure and cardiovascular mortality persisted even after excluding cerebrovascular related mortality, indicating that the association was not mainly driven by cerebrovascular diseases.

In the tract specific analyses, we observed that MD was more prominently associated with mortality than FA. The disparity in associations between FA, MD and mortality might indicate that FA and MD reflect different pathophysiologies. The exact processes underlying the changes in FA and MD are still not known. However, it is hypothesized that FA is dominated by tract coherence and axonal loss, and MD by (volume of) interstitial or extracellular fluid.[31,32] Besides a biological difference, MD seems to be a more sensitive diffusion-MRI measure in regions with crossing tracts.[33]

The associations found with MD were widespread in the brain, but more apparent to the association tracts, which may be explained by the variable susceptibility to vascular damage between different tracts. Previous research has shown that there is a differential vulnerability of white matter tracts in aging. The association tracts are more prone to insult and degeneration in later life; however, the exact mechanism explaining this differential sensitivity remains unclear.[13,14] A possible explanation could be the location of the association tracts in watershed areas, making these tracts more vulnerable to insult and vascular damage leading to faster degeneration.

Strengths of this study include the large sample size and its population-based design. Furthermore, the availability of extensive data on cardiovascular risk factors and macrostructural MRI-markers enabled us to control for potential confounders. Limitations of this study should be acknowledged. In the tract-specific analysis we used median FA and MD and this disposes spatial information compared to a voxel-based technique. Also, varying field of views relative to the cerebellum resulted in less accurate measurements and therefore less reliable conclusions on brain stem tracts. The diffusion tensor model in regions with crossing fibers can result in unreliable diffusion-MRI measures particularly for FA. In contrast to FA, MD seems to be a more reliable measure in crossing fiber regions.[33] Although we used probabilistic tractography, and excluded tracts veering off into neighboring tract, our results with FA might have been influenced in crossing fiber regions. Furthermore, we computed tract-specific volumes to correct analyses for tract-specific atrophy and for partial volume effects in the segmentations. However, we cannot totally rule out the influence of partial volume averaging of CSF on our results. Follow-up time for cause-specific mortality was shorter than mortality from all causes; this might have led to an underestimation of the association between

white matter integrity and cause-specific mortality. Although our findings were independent of several cardiovascular risk factors, roles of other potential confounders such as physical activity, dietary factors, inflammation, and carotid disease on the association between white matter integrity and mortality need to be further tested.

Our findings suggest that the adverse effects of impairments in cerebral white matter integrity, even at early stages, are not limited to neurocognitive outcomes and they can also put individuals at higher risk of mortality especially from cardiovascular causes. This might call for further studies unravelling the mechanisms behind the link between white matter integrity and survival.

CHAPTER REFERENCES

1. Chanraud S, Zahr N, Sullivan EV, Pfefferbaum A. MR diffusion tensor imaging: a window into white matter integrity of the working brain. *Neuropsychology review.* 2010;20(2):209-225.
2. Fields RD. White matter matters. *Scientific American.* 2008;298(3):42-49.
3. de Leeuw FE, de Groot JC, Achten E, et al. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *Journal of neurology, neurosurgery, and psychiatry.* 2001;70(1):9-14.
4. Debette S, Beiser A, DeCarli C, et al. Association of MRI markers of vascular brain injury with incident stroke, mild cognitive impairment, dementia, and mortality: the Framingham Offspring Study. *Stroke; a journal of cerebral circulation.* 2010;41(4):600-606.
5. Henneman WJ, Sluimer JD, Cordonnier C, et al. MRI biomarkers of vascular damage and atrophy predicting mortality in a memory clinic population. *Stroke; a journal of cerebral circulation.* 2009;40(2):492-498.
6. Sabayan B, van der Grond J, Westendorp RG, van Buchem MA, de Craen AJ. Accelerated progression of white matter hyperintensities and subsequent risk of mortality: a 12-year follow-up study. *Neurobiology of aging.* 2015.
7. de Groot M, Verhaaren BF, de Boer R, et al. Changes in normal-appearing white matter precede development of white matter lesions. *Stroke.* 2013;44(4):1037-1042.
8. Gordon BA, Najmi S, Hsu P, Roe CM, Morris JC, Benzinger TL. The effects of white matter hyperintensities and amyloid deposition on Alzheimer dementia. *Neuroimage Clin.* 2015;8:246-252.
9. Windham BG, Deere B, Griswold ME, et al. Small Brain Lesions and Incident Stroke and Mortality: A Cohort Study. *Ann Intern Med.* 2015;163(1):22-31.
10. Ikram MA, Vernooij MW, Vrooman HA, Hofman A, Breteler MM. Brain tissue volumes and small vessel disease in relation to the risk of mortality. *Neurobiology of aging.* 2009;30(3):450-456.
11. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *The Lancet. Neurology.* 2010;9(7):689-701.
12. Fazekas F, Wardlaw JM. The origin of white matter lesions: a further piece to the puzzle. *Stroke; a journal of cerebral circulation.* 2013;44(4):951-952.
13. Bender AR, Volkle MC, Raz N. Differential aging of cerebral white matter in middle-aged and older adults: A seven-year follow-up. *Neuroimage.* 2016;125:74-83.
14. Kochunov P, Williamson DE, Lancaster J, et al. Fractional anisotropy of water diffusion in cerebral white matter across the lifespan. *Neurobiol Aging.* 2012;33(1):9-20.
15. Hofman A, Brusselle GG, Darwish Murad S, et al. The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol.* 2015;30(8):661-708.
16. Ikram MA, van der Lugt A, Niessen WJ, et al. The Rotterdam Scan Study: design update 2016 and main findings. *Eur J Epidemiol.* 2015;30(12):1299-1315.
17. 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(4):1151-1161.
18. Koppelmans V, de Groot M, de Ruiter MB, et al. Global and focal white matter integrity in breast cancer survivors 20 years after adjuvant chemotherapy. *Hum Brain Mapp.* 2014;35(3):889-899.
19. 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(1):71-81.
20. Adams HH, Cavalieri M, Verhaaren BF, et al. Rating method for dilated Virchow-Robin spaces on magnetic resonance imaging. *Stroke.* 2013;44(6):1732-1735.

21. de Groot M, Ikram MA, Akoudad S, et al. Tract-specific white matter degeneration in aging: the Rotterdam Study. *Alzheimers Dement.* 2015;11(3):321-330.
22. de Groot M, Vernooij MW, Klein S, et al. Improving alignment in Tract-based spatial statistics: evaluation and optimization of image registration. *NeuroImage.* 2013;76:400-411.
23. 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(3):173-185.
24. Akoudad S, Portegies ML, Koudstaal PJ, et al. Cerebral Microbleeds Are Associated With an Increased Risk of Stroke: The Rotterdam Study. *Circulation.* 2015;132(6):509-516.
25. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *American journal of human genetics.* 2004;74(4):765-769.
26. Bokura H, Kobayashi S, Yamaguchi S, et al. Silent brain infarction and subcortical white matter lesions increase the risk of stroke and mortality: a prospective cohort study. *Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association.* 2006;15(2):57-63.
27. Virtanen S, Utriainen KT, Parkkola R, et al. White matter damage of the brain is associated with poor outcome in vascular surgery patients with claudication: a pilot study. *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery.* 2014;48(6):687-693.
28. Maillard P, Seshadri S, Beiser A, et al. Effects of systolic blood pressure on white-matter integrity in young adults in the Framingham Heart Study: a cross-sectional study. *The Lancet. Neurology.* 2012;11(12):1039-1047.
29. Rosano C, Abebe KZ, Aizenstein HJ, et al. Longitudinal systolic blood pressure characteristics and integrity of white matter tracts in a cohort of very old black and white adults. *American journal of hypertension.* 2015;28(3):326-334.
30. Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress responses. *Nature reviews. Neuroscience.* 2009;10(6):397-409.
31. Maclullich AM, Ferguson KJ, Reid LM, et al. Higher systolic blood pressure is associated with increased water diffusivity in normal-appearing white matter. *Stroke.* 2009;40(12):3869-3871.
32. Vernooij MW, Ikram MA, Vrooman HA, et al. White matter microstructural integrity and cognitive function in a general elderly population. *Arch Gen Psychiatry.* 2009;66(5):545-553.
33. Jeurissen B, Leemans A, Tournier JD, Jones DK, Sijbers J. Investigating the prevalence of complex fiber configurations in white matter tissue with diffusion magnetic resonance imaging. *Hum Brain Mapp.* 2013;34(11):2747-2766.

Table e-1. Baseline characteristics of included and excluded participants

Baseline characteristics	Participated N= 4294	Not participated N=1136	P-value
Age, years	63.6 (11.0)	68.0 (12.2)	<0.001
Men	1906 (44.4)	470 (41.4)	0.128
Systolic blood pressure, mmHg	139.0 (21.5)	144.6 (24.5)	0.017
Diastolic blood pressure, mmHg	83.1 (10.8)	83.6 (11.8)	0.548
Antihypertensive medication	1463 (34.1)	555 (48.9)	0.005
Total cholesterol, mmol/l	5.5 (1.0)	5.3 (1.1)	<0.001
HDL cholesterol, mmol/l	1.4 (0.4)	1.4 (0.4)	0.009
Lipid-lowering medication	1014 (23.6)	368 (33.2)	<0.001
Smoking			
Current	878 (20.4)	236 (21.3)	<0.001
Former	2060 (48.0)	545 (48.0)	
History of coronary heart disease	256 (6.0)	105 (9.2)	0.100
Diabetes mellitus	393 (9.2)	145 (12.8)	0.009
Body mass index, kg/m^2	27.4 (4.1)	27.9 (5.1)	<0.001

Table e-2. The association of white matter microstructural integrity and mortality excluding participants with interim stroke and dementia

	Total mortality	
	Hazard ratio* (95% CI) N=4219 (198)	p value
Fractional anisotropy		
Model I	1.35 (1.17, 1.56)	<0.001
Model II	1.31 (1.14, 1.51)	<0.001
Model III	1.27 (1.09, 1.49)	0.003
Mean diffusivity		
Model I	1.51 (1.27, 1.79)	<0.001
Model II	1.44 (1.21, 1.72)	<0.001
Model III	1.43 (1.18, 1.72)	<0.001

*Hazard ratios and 95% CI are calculated per standard deviation decrease in fractional anisotropy and per standard deviation increase in mean diffusivity.

Model I: Adjusted for age, and sex.

Model II: Model I + systolic blood pressure, diastolic blood pressure, antihypertensive medication, smoking, total cholesterol, high density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, history of coronary heart disease, *APOE-ε*4 allele carrier, and body mass index.

Model III: Model I + intracranial volume, white matter volume, logarithm of volume of white matter hyperintensities, presence of microbleeds, and presence of lacunar infarcts.

Abbreviation: CI: confidence interval, *APOE*: Apolipoprotein E.

Table e-3. The association of white matter microstructural integrity and cardiovascular mortality excluding death from stroke

	Cardiovascular mortality	
	Hazard ratio* (95% CI) N=4289 (26)	**p value**
Fractional anisotropy		
Model I	1.85 (1.29, 2.67)	0.001
Model II	1.61 (1.10, 2.35)	0.014
Model III	1.61 (1.07, 2.41)	0.023
Mean diffusivity		
Model I	1.89 (1.24, 2.88)	0.003
Model II	1.66 (1.04, 2.65)	0.032
Model III	1.63 (1.01, 2.65)	0.046

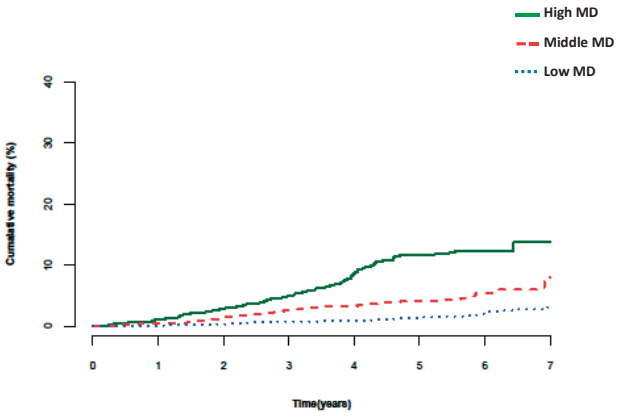
*Hazard ratios and 95% CI are calculated per standard deviation decrease in fractional anisotropy and per standard deviation increase in mean diffusivity.

Model I: Adjusted for age, and sex.

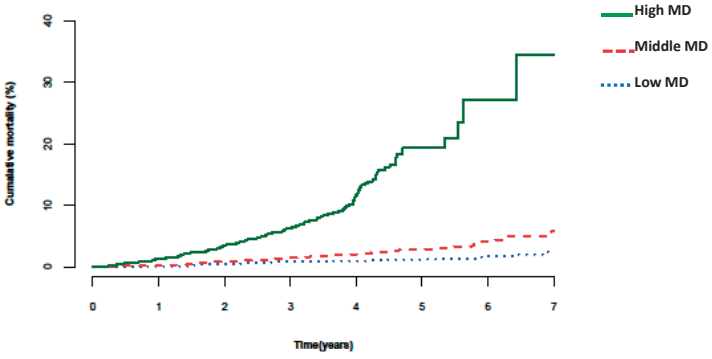
Model II: Model I + systolic blood pressure, diastolic blood pressure, antihypertensive medication, smoking, total cholesterol, high density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, history of coronary heart disease, *APOE-ε*4 allele carrier, and body mass index.

Model III: Model I + intracranial volume, white matter volume, logarithm of volume of white matter hyperintensities, presence of microbleeds, and presence of lacunar infarcts.

Abbreviation: CI: confidence interval, *APOE*: Apolipoprotein E.



A) Fractional anisotropy



B) Mean diffusivity

Figure e-1.

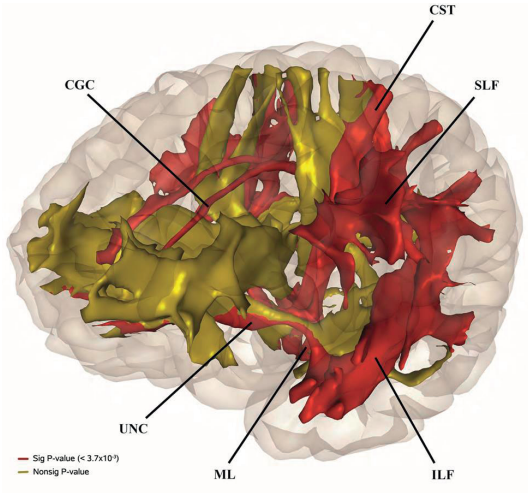


Figure e-2.