# General discussion

## MAIN FINDINGS AND INTERPRETATION

### General Discussion

The main objectives of my thesis were to study determinants of white matter microstructural changes as assessed with Diffusion-Tensor-Imaging (DTI), and to study the role of white matter microstructural changes in age related brain diseases such as cognitive decline, dementia, and neurovascular disease.
In this chapter, I will first discuss my main findings and I will consider possible pathways underlying the found associations and additionally some methodological considerations. Next, I will discuss the implications of the findings with respect to clinical practice and finally I will discuss future perspectives.

### Main findings

#### Determinants of white matter microstructural integrity

**Chapter 2** in this thesis describes the different determinants of white matter microstructural changes globally but also in specific tracts. I started with a longitudinal analysis for the establishment of reference values of change in DTI-measures in aging. Since the brain is not an isolated organ but is connected with all other organs in our body, I was also interested in the systemic influences on the brain by different organ systems, such as the kidneys, the lungs and the thyroid gland. To explore whether microvascular pathology could be one of the possible pathways leading to lower white matter microstructural integrity I also studied the association between retinal vessels and microstructural changes in this chapter.
I found lower white matter microstructural integrity in most parts of the brain normal-appearing white matter after two years of follow-up in normal aging. Also, reduced kidney function associated with lower white matter microstructural integrity throughout the brain. Retinal vessel diameters was linked to white matter microstructure by means that larger venules and narrower arteries associated with a poorer white matter microstructural integrity. Reduced lung function was linked to lower white matter microstructural integrity in particular in the association tracts. In the last chapter I found an age-dependent association between thyroid hormones and white matter microstructural integrity: in younger individuals higher levels of free thyroxine (FT4) were associated with larger total brain volumes (mainly white matter volume) and a better white matter microstructural integrity and in older persons higher FT4 associated with smaller total brain volumes and a poorer white matter microstructural integrity.
The results reported in **chapter 2** indicate that aging and systemic influences of (subclinical) disease outside the brain are consistently associated with reduced white

matter microstructural integrity. What underlies these associations? There are various potential pathways, supported by the different chapters in my thesis.

First, a vascular etiologic pathway has often been hypothesized.[1] Vascular brain pathology may lead to a reduction in white matter perfusion, for example due to impaired autoregulation, resulting in white matter damage.[2-5] Another mechanism may be that the presence of vascular factors, not causing direct damage, interact with another degenerative process and worsen pathologic effects.[6] Yet another possibility is that shared vascular risk factors such as hypertension and diabetes explain the association between (subclinical) disease outside the brain and subclinical brain changes. This latter mechanism is especially feasible in situations in which small vessels for example in the kidney or retina and brain are similar with anatomical and hemodynamic similarities.[7] My findings in **chapter 2.2** indeed confirmed a role of vascular factors. The association between kidney function and cerebral white matter microstructure attenuated after adjusting for cardiovascular risk factors, indicating that the association was partly driven by cardiovascular factors. Furthermore, I found effect-modification by hypertension in the association between kidney function and lower white matter microstructural integrity. In addition, my findings in **chapter 2.3** in which retinal vessel calibres associated with white matter microstructural integrity, suggest a microvascular pathway underlying the white matter microstructural changes. However, both in **chapter 2.1**, focussing on aging and white matter microstructure, and in **chapter 2.5** assessing the relation of thyroid hormones and brain morphology, adjusting for cardiovascular risk factors did not change the estimates marginally. This may be explained by residual confounding (e.g. unmeasured factors, age-specific effects of vascular factors or subclinical vascular factors or a genetic predisposition). However, a non-vascular pathway should be considered as well. For example, free radical injury has been suggested to associate with white matter changes on DTI.[8] This could be one of the alternative pathways explaining the association of thyroid function with lower white matter volume and lower white matter microstructural integrity in elderly. Oxidative stress has also been proposed as one of the possible factors in the pathophysiology of age-related diseases such as dementia.[9,10] Alternatively, direct toxic influences on the brain for example due to high thyroid hormone levels could underlie some of the found associations. Hypoxia, resulting in metabolic decreases and decline of cerebral perfusion is another possible pathway causing morphologic brain changes,[11] as we suggested in **chapter 2.4** in which reduced lung function was associated with lower white matter microstructural integrity. Also, systemic inflammation which may induce chronic inflammation of the vessel wall leading to lower white matter microstructural integrity is another potential mechanism leading to white matter microstructural damage as described in **chapter 2.4**.[12,13]

Apart from all above mentioned possible pathways, a more complex, multifaceted pathway needs to be considered in which there is an interplay between vascular factors, hypoxia, and inflammation.[14] Future research can help to elucidate these possible pathways. Longitudinal analyses are needed to draw conclusions on directionality of associations. More in-depth measurements of cerebral perfusion and autoregulation may shed more light on the hypotheses on hypoperfusion and hypoxia. Mediation analyses with the inclusion of possible mediators such as inflammatory markers can help to study disease causal pathways. Also, hypothesis-free genome-wide association studies can uncover novel genes and pathways involved in age-related brain pathology.

**White matter microstructural integrity and cognitive decline, mild cognitive impairment, dementia and hearing loss**

When focusing on white matter microstructure and age-related brain diseases, my most important finding was that white matter microstructural integrity both globally and in various tracts related to cognitive function and risk of dementia, and that this association was more pronounced for some tracts than for others. I found that reduced white matter microstructural integrity throughout the brain, but mainly in the association and projection tracts related to poorer cognition. In a longitudinal analysis, I found a similar pattern, again that in particular the association, projection and limbic system tracts related to risk of dementia and with more pronounced cognitive decline. In addition, the white matter microstructure of in particular the association tracts was related to mortality. The found associations remained after adjusting for macrostructural white matter changes such as white matter atrophy and white matter hyperintensity and thus support the hypothesis that white matter microstructural integrity is an earlier and potentially more sensitive marker of white matter damage.

**What underlies these associations?**

The role of vascular factors in the association of white matter microstructural integrity and age-related brain diseases seems evident. Indeed, in **chapter 3.1**, I found that cardiovascular risk factors were related to mild cognitive impairment, a transitional stage between aging and dementia. Besides cardiovascular risk factors, also markers of small vessel disease such as white matter hyperintensities were related to MCI, and these results indicate the important role of vascular factors in early phases of age-related diseases such as dementia. Also, in an additional analysis in **chapter 3.3** (data not published) we found overall and age-specific associations of cardiovascular risk factors (i.e. hypertension, hypercholesterolaemia, current smoking, diabetes, and body mass index) with global white matter microstructural integrity from middle age on as depicted in **Figure 1**. In addition, low white matter microstructural integrity was mainly related to cardiovascular mortality as described in **chapter 3.5**. However, some

of the found associations did not attenuate after adjustments for cardiovascular risk factors, as we described in **chapter 3.2 and 3.3** in which we focused on tract-specific white matter microstructural integrity and the association with cognition and dementia. Also, adjusting for cardiovascular risk factors did not change the estimates substantially studying white matter microstructural integrity and hearing loss in **chapter 3.4**. Hearing loss possibly adds to the cognitive load of a vulnerable brain leading to white matter microstructural brain changes.[15] Also other mechanisms, as we described above, should be considered in contributing to the found associations and this need to be further investigated in future research.

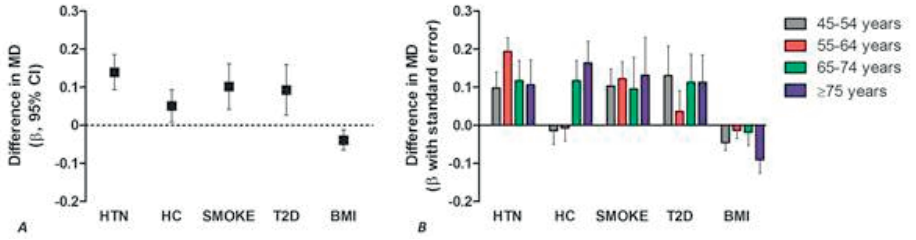


**Figure 1**

Cognitive performance consists of developmental and degenerative components.[16] Recently, the highly polygenic architecture of cognition was partly elucidated by the identification of genetic variants in ninety-nine independent loci (Davies et al, in press). We aimed to elucidate the associations of the genetic variants underlying cognition with cognitive decline, daily functioning, dementia, parkinsonism and stroke. Brain changes may be an intermediate between the genetic variants and the different endpoints and therefore we also studied the link between the genetic variants and brain imaging markers including DTI-measures globally and tract-specific. A higher polygenic score (PGS) was associated with cognitive decline. No association was found with daily functioning, the incidence of dementia, parkinsonism or stroke. A higher PGS was related to a larger intracranial volume and higher educational attainment and associated with a better white matter microstructure in the posterior thalamic radiation, inferior-fronto-occipital fasciculus and in the parahippocampal part of the cingulum. These white matter tracts have been associated with cognitive performance previously.[17] This study suggests that the studied genetic variants associated with cognition represent both developmental and degenerative components of cognitive performance. This indicates that the results in **chapter 3.2 and 3.3** assessing the relation of white matter microstructural integrity and cognition and dementia, apart from neurodegeneration can also be partly explained by developmental influences.

To investigate the potential clinical use of advanced imaging markers we aimed to evaluate a previously proposed prediction tool, namely the Disease State Index

(DSI)[18], to predict cognitive decline using (advanced) imaging and non-imaging features, including global and tract-specific DTI -measures. Though for several of the brain imaging markers that were introduced in the DSI, independent associations with risk of cognitive decline and dementia had been described,[19-21] their added value in the prediction model was disappointingly low. Best performance of the prediction of global cognitive decline was obtained using only age as input feature. Adding additional input features to the DSI did not improve prediction of global cognitive decline in the general population. This is in concordance with a previous population based study, concluding that MRI markers on top of age and other conventional risk variables did not improve the prediction of all cause dementia.[22] This indicates that more work is needed in the domain of disease prediction modeling of dementia and other age-related brain diseases, and that specifically in the field of imaging markers we may need to focus more on either fine-grained advanced imaging features such as functional MRI measures, multimodality imaging[23] or the use of other analytical approaches such as deep learning algorithms[24] in medical image analysis.

**Methodological considerations related to studies in this thesis**

**Population-based study design**

The studies discussed in this thesis were conducted within the Rotterdam Study, a prospective population based cohort study starting in 1990, including inhabitants of the district Ommoord of the city Rotterdam, aged 45 years and over, designed to study determinants and prognosis of chronic diseases in elderly.[25] Participants are considered to be a random sample from the general population, which reduces the possibility of selection bias and thus increases generalizability of the results. However, it must be noted that the participants of the Rotterdam Study are mostly Caucasians, making the results only generalizable to similar populations. In order to participate, in particular for the MRI, inhabitants of Ommoord needed to visit the research centre. Therefore, the healthy volunteer effect (another form of selection bias) cannot be ruled out, since it is likely that participants with poorer health did not participate. The inclusion of relatively healthy persons however will most likely lead to an underestimation of the found effect estimates. The entire cohort of the Rotterdam study is under continuous surveillance for outcomes such as dementia and mortality through electronic linkage of the study database with medical records from the general practitioners and the regional institute for outpatient mental health care. This minimizes loss to follow-up and reduces the healthy volunteer effect. The longitudinal analyses presented in this thesis, studying the association between white matter microstructure and dementia or mortality, are therefore less likely influenced by selection bias.

Differential misclassification or measurement error is a form of information bias which might have influenced our resuls, since it is likely that DTI-measures were less accurately measured in older persons, in persons with preclinical dementia or brain atrophy due to motion artifacts.

Confounding, a situation in which an association between exposure and outcome is distorted by the presence of another third variable[26], can be prevented by restriction, stratification or adjustment. In this thesis we performed multivariable adjusted regression models to account for several potential confounders, mainly vascular factors. Yet, variables included in the model that do not meet the criteria for potential confounders (e.g. intermediates) can introduce over-adjustments bias.[27] This may have influenced our results, since white matter microstructural changes may be intermediates in the relation between vascular factors and different outcomes such as dementia. I present a basic model and additionally a model including vascular factors in most of the presented studies in this thesis to assess the potential role of this over-adjustments bias effect. Most of the studies described in this thesis were performed with data that was acquired cross-sectionally. Due to the cross-sectional design, no conclusions can be drawn on the directionality of causality of the associations presented in this thesis. However, **chapter 3.3** is a longitudinal study on the relation between white matter microstructural integrity and dementia and cognitive decline. To address potential reverse causality, we repeated this analysis after stepwise exclusion of the first five years of follow-up. The risk estimates did not change, therefore it is very unlikely that the associations were driven by (subclinical) dementia cases (reverse causality).
The studies described in this thesis were conducted within large sample sizes and this reduces the chance on false positive findings in comparison to smaller imaging studies.

**Imaging protocol**

From 2005 onwards, MRI was implemented in the study protocol and a dedicated research scanner, undergoing a QA protocol keeping the system unchanged (no major updates or upgrades) was installed in the Rotterdam Study research center.[28] Since this is a population-based study there is a restriction of time, costs and inconvenience for the participants which needs to be balanced with the relevance and quality of the acquired imaging data. In the design of the scan protocol the time constraint, contrast and resolution requirements were taking into account. Back in 2005, when the MRI protocol was designed, the number of gradient directions was chosen to best fit the optimized protocol proposed by Jones et al.[29] taking time limits and the number of slices permitted into account. Acquisition parameters have been left unchanged since the start of the study to ensure longitudinal data stability and comparability over time. We acknowledge the fact that the spatial resolution for the diffusion acquisition used

in this thesis was relatively lower than current standards and we are aware of the lower precision to detect crossing fiber populations using 25 gradient directions. However, probabilistic tractography within the Rotterdam Study was performed with fully automated methods, publicly available methods[30] with a good reproducibility. The median R^2 of the reproducibility of the FA measurements was 0.89, in participants scanned twice within two weeks.[31] Therefore there is no reason to assume that our diffusion-weighted MRI protocol severely affected the ability to accurately reconstruct tracts of interest. We however used aggregated diffusion measures and averaging of FA and MD measures and this might discard some spatial information.

**Underlying tissue properties**

The diffusion of water molecules in the brain is dependent on multiple factors, including the underlying white matter microstructural integrity, which is believed to be predominantly determined by the alignment and diameter of white matter tracts and their myelin.[32] The diffusion profiles of water molecules in the brain, based on diffusion-weighted MRI measures, give indirect information about the underlying tissue or white matter microstructural integrity of the brain. Fractional anisotropy (FA) and mean diffusivity (MD) are the two most commonly used diffusion-weighted MRI measures. However, to what extent diffusion tensor imaging measures may be informative about the exact underlying tissue structure is still under debate. Generally it is assumed that lower FA and higher MD are associated with lower white matter microstructural integrity and there is pathological evidence that changes in these DTI-measures correlate with myelin damage and axonal loss.[32] Previously it was suggested that axial and radial diffusivity (two other DTI-measures) could disentangle whether changes in the white matter microstructure were due to axonal loss or due to myelin loss. However, the presence of other possible processes such as inflammation generates difficulties to assigning change in diffusion-MRI measures to a specific underlying pathological process (e.g. myelin loss) causing the observed lower microstructural integrity.[33] Yet based on previous research (including animal studies)[34], the found associations in this thesis, but also on the fact that white matter microstructural changes precede white matter macrostructural changes[35] we can assume that changes in diffusion-weighted MRI measures are pointing towards brain pathology of any kind.

Furthermore, it is now widely recognized that voxels with complex configurations are a challenge for DTI. The “crossing fiber problem” typically refers to the situation with two or more differentially oriented fiber bundles contributing to the diffusion-weighted MRI signal leading to incorrect DTI estimates and failure of the tractography. Previous research showed that up to 90% of the cerebral white matter voxels contain crossing fibres.[36] Interpretation of DTI-measures therefore must be done with caution. Until now the crossing fiber problem appears to be a fundamental limitation of DTI that

comes with the complexity of brain tissue, rather than a technical problem that can be overcome with a higher spatial resolution.[37]

**Differential effect and vulnerability of white matter tracts**

Previous studies found a differential vulnerability to decline for different white matter tracts and stated that the association tracts are most vulnerable to decline and aging.[38,39] Also in this thesis I found that the microstructural integrity of primarily the association tracts was associated with the studied determinants and outcomes in this thesis. A possible explanation could be the location of the association tracts in watershed areas, making these tracts more vulnerable to vascular damage leading to more pronounced loss of white matter microstructural integrity. Tract-specific analyses may thus have added value in providing novel etiological insight into structural brain pathology, the location of brain microstructural damage with relation to age-related brain outcomes and may help to unravel underlying pathways. In this thesis I also found that different association tracts were related to different outcomes. For example the white matter microstructure of association tracts related to hearing impairment were in particular the superior longitudinal fasciculus and the uncinate fasciculus, while the white matter microstructure of the inferior-fronto-occipital fasciculus associated most strongly with cognitive performance. This possibly points towards disease-specific effects which can be detected using tract-specific analyses, with interesting possibilities towards risk stratification and early disease prevention.

However, future studies are needed to replicate our findings to see whether these tract-specific differences are true findings initiated by disease or initiated by technical issues (e.g. the association tracts are larger tracts, making tractography less sensitive to partial volume effects compared to smaller tracts).[40]

**Clinical implications and future research**

In this thesis I contributed to the establishment of reference values of change in DTI-measures in aging. Furthermore, I highlight the added value of (tract-specific) DTI-measures over conventional MRI markers to study determinants and outcomes of white matter disease. This further supports the observation that macrostructural markers only represent the tip of the iceberg of the white matter damage. More etiological knowledge can be obtained using (tract-specific) DTI. I found tract-specific differences; among the 15 white matter tracts, the association tracts most often related to age-related brain outcomes such as cognitive performance. This may have clinical implications, since knowledge on tract-specific effects on cognition may inform clinicians to predict which cognitive domains may be most affected depending on the location of (focal) white matter damage, e.g. in chronic pathology or in an acute setting such as an acute stroke. Also, given the importance of recognizing pathways leading to

age-related brain diseases our results may aid in developing new biomarkers and may lead to better recognition of persons at risk.

Reduced kidney function and lung function were both associated with cognitive impairment. Based on the results presented in this thesis we suggest that white matter microstructural changes possibly mediate the association between different determinants (e.g. kidney function and lung function) and age-related brain diseases such as cognitive impairment. The traditional approach to mediation analysis is to adjust for the mediator in regression analysis. However, recently alternative analytic methods have been introduced to improve the interpretation of mediation analyses.[41] Future research should incorporate these methods to investigate whether changes in white matter microstructural integrity actual mediate the association of several determinants with age-related outcomes.

Although several associations were found between white matter microstructure and age-related brain diseases, this does not necessarily indicate that these markers are of value in disease prediction as shown in the DSI study we performed. Thus, a logical next step before clinical implementation of imaging markers is to investigate the added value of imaging markers including DTI for the prediction of age-related diseases. Prediction models other than the DSI tool, in the general population but also in specific patient populations, need to be tested and used as reference model to verify whether imaging markers including DTI-measures when added to the model can improve prediction of age-related diseases such as dementia.

Also, prediction of age-related diseases is calling for more advanced analytical approaches such as deep learning algorithms or machine learning methods, since extracted, often aggregated imaging markers so far do not improve prediction performance on top of age.

**Concluding remarks**

In this thesis I contributed to the establishment of reference values of change in DTI-measures in aging. I investigated different determinants of white matter microstructural changes, and studied the relation of white matter microstructure with age-related brain diseases. In this chapter I gave an overview of the main results, discussed methodological considerations regarding the performed studies and mentioned some clinical implications and directions for future research. The white matter microstructure of the association tracts seems to be most sensitive to decline. Although several associations were found between white matter microstructure and age-related brain diseases, the use of (global and tract-specific) DTI-measures to support clinical decision-making and prediction of age-related brain diseases needs further steps, which should focus on

prediction modelling, inclusion of more fine-grained imaging features, or the use of other analytical and methodological approaches.

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