SUMMARY AND DISCUSSION
In this thesis, we assessed structural MRI changes in predementia Alzheimer’s disease (AD) to gain insight into pathological mechanisms occurring early in the disease course. A better understanding of these early changes could aid in improving AD diagnosis before the onset of dementia, as well as inform on prognosis in non-demented subjects. These are necessary conditions for the development and implementation of secondary prevention strategies in preclinical and prodromal AD.

The main findings of this thesis are:

- Structural MRI changes are already detectable in the preclinical stage of AD;
- Structural MRI can be used as a prognostic marker for cognitive decline in preclinical and prodromal AD;
- Grey matter network measures have added value beyond volumetric measures for early diagnosis and prognosis of AD;
- Prodromal AD has distinct atrophy subtypes, which are associated with specific trajectories of cognitive decline.

In this chapter, we summarize the findings from the different studies in this thesis and place them into the context of existing literature and ongoing research projects. We then elaborate on some of the methodological considerations that should be taken into account when reading this thesis. We finish by discussing the implications of our results and provide recommendations for future research.

**NEUROIMAGING MARKERS FOR DIAGNOSIS AND PROGNOSIS IN PREDEMENTIA AD – LITERATURE STUDIES**

In the first two chapters of this thesis, we have reviewed the current literature on the use of neuroimaging biomarkers (PET and MRI) in the predementia stage of AD. In chapter 1 we review the available evidence for use of neuroimaging in clinical trials in preclinical and prodromal AD, for subject selection, stratification, as outcome measure and to monitor trial safety. From the available literature, we conclude that amyloid PET, an in vivo marker of fibrillary amyloid plaques, is appropriate for subject selection, and to monitor treatment target engagement (in the case of anti-amyloid therapies). Amyloid PET, however, cannot be used to predict the rate of cognitive decline within the 2-5 year time frame of a clinical trial. Volumetric measures derived from structural MRI can be used for trial enrichment as they can identify subjects at risk of imminent cognitive decline. Longitudinal hippocampal atrophy rates can be reliably derived from structural MRI and are useful in tracking disease
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progression and as outcome measure. More advanced MRI techniques, such as diffusion tensor imaging or functional MRI, require further validation in preclinical and prodromal AD.

In chapter 2 we performed a literature review on the use of hippocampal atrophy as an early diagnostic and prognostic marker in routine clinical practice in the assessment of individuals with mild cognitive impairment (MCI). Hippocampal atrophy is part of the research criteria for the diagnosis of AD in individuals with MCI [1]. Although these criteria were developed for research purposes, they now affect clinical practice [1,2]. However, evidence for the use of hippocampal atrophy for a diagnosis of AD in the MCI stage had not been systematically assessed [3]. In the oncology field, research on cancer biomarkers has been systematized based on a framework borrowed from drug development [4]. The Geneva Roadmap [5] has adapted this framework to systematically assess AD biomarkers in the MCI stage [6]. We conclude that although hippocampal atrophy is a validated biomarker of AD, it has limited specificity to distinguish underlying AD pathology from other neurodegenerative diseases. Furthermore, most studies on the accuracy of hippocampal atrophy to predict progression from MCI to AD dementia have been performed on research samples with short follow-up. Longer, prospective studies in clinical MCI populations are necessary. Other issues include insufficient progress on the optimization and standardization of measurement algorithms for hippocampal volume and the definition of universal cut-off values for abnormality. As part of the Geneva Roadmap also other biomarkers were reviewed within the same framework: episodic memory [7], cerebrospinal fluid (CSF) measures [8], amyloid PET [9], [18F]FDG-PET [10], and [123I]Ioflupane and [123I]MIBG imaging [11].

VOLUMETRIC AND GREY MATTER NETWORK CHANGES IN PREDEMENTIA AD

The next studies were performed to gain a better understanding of structural brain changes in predementia AD. In chapter 3 we showed that risk factors for amyloid aggregation and AD dementia, namely a family history of AD dementia and the presence of an Apolipoprotein E ε4 allele, are already associated with grey matter atrophy in various brain regions in cognitively normal subjects aged 39-79 years (mean age 57) using voxel-based morphometry. Subjects with these risk factors showed grey matter atrophy in the precuneus, striatum and insular regions compared to subjects without these risk factors. Longitudinal data is necessary to determine whether these findings are associated with progression to AD dementia.
Next, we assessed the association between structural MRI measures and amyloid pathology in non-demented subjects. In chapter 4 we assessed the association between amyloid pathology and easily obtainable MRI measures (visual rating, volumetric measures and cortical thickness) in a large multicentre cohort of cognitively normal subjects and subjects with MCI. We found that amyloid pathology was associated with lower subcortical volumes (hippocampus, amygdala, accumbens) and reduced cortical thickness in AD-signature regions (Figure 1). These effects were more pronounced in prodromal AD compared to preclinical AD. We then built an automated classifier based on clinical and imaging variables, which could identify the presence of amyloid pathology with a moderate level of accuracy in non-demented subjects. The results from this study might be of interest for trial designers, as the classifier could be used to pre-screen subjects for enrolment into secondary prevention studies.

Structural brain changes in neurodegenerative diseases may not occur randomly, but have been hypothesized to follow changes in brain networks [12]. One way to measure brain networks is by examining patterns of coordinated grey matter morphology, which can be derived from structural MRI. In chapter 6 and chapter 7 we showed that amyloid pathology is associated with grey matter network changes in two separate cohorts of cognitively normal individuals. In the first cohort, we found that lower levels of CSF amyloid beta 1-42, indicative of more amyloid pathology, were associated with lower connectivity density, clustering coefficient, and higher path length values, suggesting a less-efficient network organization. In another cohort, we found that higher global amyloid standardized uptake value ratio (SUVr) measured with PET, indicative of more amyloid pathology, was associated with lower clustering coefficient and lower small world values. The regions affected were similar in the two studies (Figure 1). Together, these two studies indicate that increased amyloid aggregation is associated with alterations in grey matter networks that are indicative of incipient network breakdown, as seen more severely in AD dementia. These grey matter network disruptions might be part of the earliest pathological changes in AD and occur before gross atrophy. Therefore, they could be a sensitive biomarker for early diagnosis of AD.
Figure 1: Summary of results from chapters 4 to 8.

Upper panel: association between amyloid pathology and structural MRI measures of cortical thickness (left), voxel-wise grey matter volume (middle) and grey matter network clustering coefficient (right) in cognitively normal subjects (upper row) and subjects with mild cognitive impairment (lower row). Lower panel: structural MRI measures predictive of rate of cognitive decline in amyloid positive subjects.
MRI AS PROGNOSTIC MARKER IN PRECLINICAL AND PRODROMAL AD

As described in chapter 1, non-demented subjects with evidence of amyloid pathology in CSF or on PET are at an increased risk of cognitive decline. However, since amyloid reaches a plateau relatively early in the disease course [13–15], it has limited prognostic value for the time to onset of dementia. Previous studies have shown that hippocampal volume is associated with time to progression to dementia in prodromal AD [16,17]. However, other brain regions may also be valuable for the prediction of clinical progression [18–20]. In chapter 5 we implemented a voxel-wise survival analysis to identify in an unbiased way brain regions where decreased grey matter volume is associated with time to dementia in prodromal AD. We found a widespread pattern of decreased grey matter volume, beyond the hippocampal region, to be predictive of time to progression to dementia (Figure 1). Atrophy in these regions, however, was also associated with time to progression to dementia in MCI subjects without amyloid pathology.

In chapter 8 we showed that grey matter network changes are associated with the rate of clinical progression in preclinical and prodromal AD. More specifically, lower values for degree, clustering coefficient, normalized clustering, normalized path length, and the small-world property, indicative of a more randomly organized network, predicted faster clinical progression. Regional analyses of clustering coefficient showed that the regions predictive of rate of progression were similar to those regions that were associated with amyloid pathology in preclinical AD (Figure 1).

Taken together, these studies show that both in preclinical and prodromal AD, changes in grey matter volume and grey matter network measures can be observed, which are predictive of faster clinical progression. These findings support the notion that disruptions in brain network measures and brain atrophy underlie cognitive decline. These markers can be useful to identify subjects who will show rapid clinical progression, which could then be included in clinical trials.

HETEROGENEITY IN ATROPHY PATTERNS

In chapter 9 we studied heterogeneity in atrophy patterns in AD dementia, and assessed whether this heterogeneity can already be detected in an earlier disease stage. In patients with AD dementia, we identified four atrophy subtypes that were associated with distinct clinical, biomarker and cognitive profiles. We then classified subjects with prodromal AD into these atrophy subtypes, and we found the same distinction in clinical, biomarker
and cognitive profiles. These findings suggest that the observed heterogeneity in atrophy patterns has a biological basis, which is already manifesting in the predementia stage of AD. Possibly, other pathological processes underlie these different disease subtypes, which might warrant different treatment approaches. Moreover, in subjects with prodromal AD, atrophy subtype predicted the rate of progression to dementia and was associated with decline in specific cognitive domains. Recognizing this phenotypic variation in atrophy subtypes in prodromal AD could be used to improve patient care, as it may improve individual prognosis.

**MRI MARKERS OF VASCULAR DAMAGE**

As described in chapter 1, a plethora of vascular changes can be detected with MRI, including white matter hyperintensities (WMH) on T2-weighted fluid-attenuated inversion recovery (FLAIR) MRI. Previous studies have shown that cerebrovascular changes may have an additive effect on neurodegeneration and accelerate cognitive decline and progression to dementia [21,22]. A study in autosomal-dominant AD even suggested WMH to be part of the pathophysiological cascade of AD, as these could be detected years before the onset of symptoms [23]. WMH are also frequently detected in cognitively normal subjects, and the prevalence and load increases with age. In chapter 10 we describe the association between WMH and vascular risk factors in a sample of cognitively normal elderly monozygotic twins. We show that the amount and regional distribution of WMH is highly similar within twin pairs, suggesting a genetic background for the occurrence and regional distribution of WMH. We found that up to 83% of the relation between vascular risk factors and WMH could be explained by shared genetic effects, meaning that the association between both traits is driven by genes that influence both vascular risk factors and WMH. In agreement with other studies, vascular risk factors, however, only explained a small proportion of the variance in WMH [24–26]. This suggests that factors other than small vessel disease contribute to the occurrence of WMH, such as Wallerian degeneration [27], which could be secondary to cortical AD pathology.

**METHODOLOGICAL CONSIDERATIONS**

Several methodological issues have to be considered when interpreting the results of this thesis.
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Cohorts

In this thesis, we have made use of existing data from various cohorts. The use of different data sources allowed us to replicate our results across independent cohorts. For example, the association between amyloid pathology and grey matter network measures in cognitively normal subjects was examined in two independent cohorts (chapter 6, 7) and we identified atrophy subtypes in three independent AD dementia patient samples in chapter 9.

The CITA (chapter 3, 6), INSIGHT (chapter 7) and PreclinAD monozygotic twin (chapter 10) studies were single-centre research cohorts in which all data was collected using the same protocols. In these studies, cognitively normal subjects agreed to undergo various examinations solely for research purposes including extensive neuropsychological testing, MRI and lumbar puncture or PET, and might therefore not be representative of the general population. Subjects from the INSIGHT cohort were selected based on the presence of subjective memory complaints to enrich the cohort for preclinical AD [28], also limiting the generalizability to a broader population. The Amsterdam Dementia Cohort (chapters 5, 8, 9) consists of subjects attending the memory clinic of the VU University Medical Centre. In this cohort data was collected as part of clinical routine over a long period of time, during which protocols and diagnostic criteria evolved and new scanners were implemented. This might have introduced heterogeneity in the data set, but better reflects clinical practice. We have corrected for scanner in all statistical analyses. The EMIF-AD MBD study (chapter 4) was composed of data from multiple existing European cohorts including both population-based and memory-clinic based studies, leading to a lot of variability in the dataset caused by centre effects. Prior to pooling the datasets, clinical and neuropsychological data were harmonized [29], and biomarker data analysis was performed centrally where possible. Moreover, we corrected for centre in the statistical analysis. The heterogeneity in this cohort can also be considered a strength of the study, as it supports the robustness of the results.

Amyloid assessment

During life, amyloid pathology can be assessed using PET or CSF. In general, both measures show good concordance, although this may be slightly lower in the predementia stage of AD [30]. Both methods provide different types of information. The ligands used for PET acquisition bind to the amyloid beta in fibrillary plaques [31], and thereby provide a direct measure of amyloid plaque deposition in the brain. In CSF, soluble amyloid beta 1-42 is measured in monomers and as complex with other proteins and oligomers, which are thought to decrease in response to the retention of amyloid beta into plaques in the brain [32].
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Some studies have suggested that amyloid alterations may be detected somewhat earlier in CSF than on PET [30,33]. As there is a wider variability in CSF amyloid measures between subjects within the normal range, and amyloid PET suffers from floor effects, amyloid markers in CSF might be more sensitive in the early stage. This distinction is especially relevant in cognitively normal subjects who have little amyloid aggregation. In the studies presented in this thesis, we have used both types of amyloid measurements. Some of the discrepancies in findings between the studies could be explained by this difference in measuring technique (e.g. chapters 6, 7).

Another issue with amyloid pathology measurements is the definition of a threshold for abnormality, which is commonly based on an optimal separation of subjects with AD dementia from cognitively normal subjects. In chapter 4, we have compared structural MRI parameters between amyloid positive and amyloid negative subjects. However, in chapter 6 we found that already within the normal range, CSF amyloid levels are associated with structural MRI changes. This subthreshold amyloid pathology has also been associated with increased risk of cognitive decline [34], brain atrophy [35], and increased rate of amyloid accumulation [15] in other studies. When classifying subjects as amyloid positive or negative based on a specific threshold, subjects approaching the threshold are still classified as amyloid negative, whereas they could already be in the earliest stage of AD. For future studies, it might be more informative to categorize subjects into clearly negative, clearly positive and an intermediate/at risk amyloid group.

MRI: atrophy measures

In this thesis we have performed measurements of atrophy on structural MRI sequences. Volumetric measures such as hippocampal volume usually have a high test-retest reliability when the same scanner is used (test-retest variability 1-4%), or when acquisition protocols have been harmonized prior to the study [36,37]. The agreement in terms of absolute volumes varies with acquisition protocols and field strength. For example, a change in voxel size can lead to a 5% difference in hippocampal volume [38]. In some studies in this thesis we have used datasets in which different scanners and acquisition protocols were used. Although we have corrected for potential influences by adding scanner or cohort as covariate in the analysis, we cannot exclude the possibility that this variability might have had an impact on the results. This type of variability usually adds noise to the data, so is likely to result in an underestimation of the results.

Beyond volumetric measures, we have also examined voxel-based measures. An advantage of the latter is that is does not require prespecified regions to be determined, but rather allows an unbiased whole-brain data-driven analysis. A limitation of voxel-based methods is that the number of performed statistical tests is very large (typically around 300,000...
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voxels of 1.5 mm$^3$ are included in the grey matter), which requires correction for multiple comparisons. Another issue with voxel-based comparisons is the need for spatial smoothing after warping the scans from native space to a common template. The amount of smoothing is arbitrarily chosen by the investigator, but could have an impact on the results [39].

MRI: grey matter network measures

In three chapters in this thesis we have examined grey matter network measures. For these studies we derived grey matter networks at a single-subject level [40]. An advantage of this method in comparison to group level approaches to derive structural networks [41], is that single-subject network measures can be compared with inter-individual measures in other biomarkers, cognition or decline over time. A potential limitation of our method to derive networks is that it results in subject-specific network sizes and degrees. A previous study has shown that these network measures could affect other network properties and influence the results of the study [42]. Therefore, some authors opt to enforce similar network size and degree across subjects. However, these methods might also introduce bias, especially in the case of atrophic or network changes as seen in neurodegenerative diseases. In our analysis we have assessed the impact of network size and degree on the analysis and where necessary included these measures as a covariate in the analysis of more complex network measures. How to deal with networks of unequal size and degree remains an open question. Our research group is currently examining this question in more detail, which will hopefully provide answers in the near future.

IMPLICATIONS AND FUTURE DIRECTIONS

Clinical trial design

The long predementia stage of AD in which molecular, functional and structural brain changes occur provides a unique window of opportunity for intervention. Identifying subjects at risk of AD dementia is of critical importance for the design of secondary prevention studies. Biomarkers of amyloid pathology (CSF and PET) can be used to include subjects with underlying AD pathology, who could benefit from anti-amyloid therapy. However, screening a large number of potential subjects for amyloid pathology is costly and invasive, and likely to result in a large number of screen-failures. As shown in chapter 4, easily obtainable measures from structural MRI combined with demographic and clinical information could be used to pre-screen individuals for amyloid pathology.

Information derived from structural MRI can also aid in identifying those subjects that are at an increased risk of cognitive decline within a short time frame as shown in chapters 1, 5 and 8. This is especially relevant for phase three clinical trials that target cognition as
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the primary outcome. Powering intervention studies in preclinical AD on clinical outcome requires large numbers of subjects to be included [43]. MRI measures are currently already being used as secondary outcome markers to examine disease-modifying effects. It would be of tremendous value if imaging markers could officially qualify as surrogate outcome measure in preclinical and prodromal trials [43]. For this, more research is needed on translating treatment effects on imaging measures to clinical outcomes.

Furthermore, trial designers might need to consider the heterogeneous nature of AD. In chapter 9 we showed that in prodromal AD, atrophy subtypes similar to those in the AD dementia stage can be detected. The existence of these atrophy subtypes in prodromal AD subjects with different trajectories of cognitive decline may require subtype specific outcome measures, which are tailored to the expected rate of decline in different cognitive domains. Moreover, these atrophy subtypes had different biomarker characteristics in terms of CSF tau levels and co-morbid vascular pathology. This suggests that different pathological mechanisms underlie these subtypes, which might require different treatment strategies.

**Amyloid cascade hypothesis**

The research presented in this thesis was conducted within the framework of the amyloid cascade hypothesis [44–46]. This hypothesis postulates that the aggregation of amyloid pathology is a central event in the pathogenesis of AD and initiates downstream neurodegenerative brain changes, that lead to cognitive decline and eventually dementia.

Using amyloid pathology as a starting point and proxy for early stage AD, we examined MRI biomarkers to gain insight into early pathological changes, and inform on disease stage and clinical prognosis. By which mechanisms amyloid pathology might lead to downstream events, amongst which changes in grey matter network measures and brain atrophy, remains unknown. In line with other studies [47,48], we did not find a regional relationship between amyloid pathology and structural brain changes (chapters 5, 7, 9). Possibly, amyloid might exert distant, rather than local toxic effects [49], for example through disruption of connective tracts. Alternatively, other factors, downstream from amyloid beta plaque formation, drive structural brain changes. In this scenario, amyloid pathology could initiate a cascade of events, which leads to neuronal injury and resulting cognitive impairment, detached from the original trigger of amyloid pathology. Hypothesized mechanisms include tau pathology spread or inflammatory processes [50,51]. Possibly, regional tau deposits may show more clear associations with regional disruptions of brain structure and function than regional amyloid pathology [52,53]. With the advent of new tau-binding ligands for PET, which allow the visualisation of neurofibrillary tangles *in vivo* [54], the anatomical relation
between amyloid plaques, tau deposits, grey matter network changes and brain atrophy can be examined in future studies [55]. As such the variety of biomarkers at hand will offer the possibility to study the amyloid cascade in its full entirety.

**Future research**

Further validation of existing and the discovery of new biomarkers for AD remains an important goal for future research. Although in this thesis we have primarily investigated structural MRI markers, other MRI sequences could also be useful in tracking early pathological changes in AD. Advanced MRI techniques, such as arterial spin labelling and resting state functional MRI, hold promise but require further work on standardization and harmonization of acquisition and analysis techniques.

Other pathologies that may contribute to cognitive decline, such as vascular pathology, synaptic loss and inflammation [22,23,46,50], should also be further examined in the predementia stage of AD to understand their role in the pathophysiological cascade. These pathologies could partly explain the observed heterogeneity between patients with AD in the dementia and predementia stages, which might warrant different therapeutic approaches.

**CONCLUDING REMARKS**

It is an exciting time for the AD research field. The discovery of various biomarkers over the past two decades, which allows the detection of AD pathology *in vivo* and before the onset of symptoms, has resulted in a better understanding of the pathological events leading to AD dementia. Additionally, combined efforts from scientists, government funding bodies and industrial partners have resulted in an increasing number of available datasets for researchers to use, which has facilitated the discovery of new biomarkers and the validation of existing ones. In this thesis, we have used various existing datasets to identify new and validate known imaging biomarkers that can aid in the diagnosis and prognosis of AD in the predementia stage. Nonetheless we are not there yet. The translation of these findings into routine clinical practice requires further work, as evidenced by the recent American Association for Neurology clinical guidelines, which state that there are still no accepted biomarkers that predict progression to dementia in patients with MCI [56].

In parallel with our efforts to detect and track AD pathological changes in the predementia stage, ongoing clinical trials are testing disease-modifying therapies in this population [57,58]. The discovery of these potential new drugs will further increase the necessity for
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accurate diagnosis and prognosis in the predementia stage to select appropriate subjects to be treated (i.e. with molecular evidence of AD pathology and a high risk of progressing to dementia), and to monitor treatment effectiveness.
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