General discussion, implications and recommendations for future research
The general objective of this thesis was to explore further hippocampal atrophy on MRI in the spectrum from normal aging to dementia, beyond existing evidence of hippocampal atrophy as a marker for Alzheimer’s disease (AD). First, we demonstrated that hippocampal atrophy on MRI is a marker for clinical AD regardless of the age of patients, but that it lacks specificity when attempts are made to distinguish AD from frontotemporal lobar degeneration (FTLD). Second, we showed that subjects with mild cognitive impairment (MCI) are heterogeneous with regard to MRI abnormalities suggestive of Alzheimer-type and cerebrovascular pathology. Medial temporal lobe atrophy, more than small vessel disease, seems to be associated with cognitive function and specific clinical subtypes of MCI. Third, we identified predictors of accelerated rates of hippocampal atrophy in MCI, in turn indicative of accumulating Alzheimer-type pathology. Older age, poorer general cognition, the presence of an APOE e4 allele and hippocampal atrophy, all well-reported risk factors for AD, were associated with increased hippocampal atrophy rates in subjects at the early stages of cognitive decline. Finally, we demonstrated improved reliability of a new, more automated method for hippocampal atrophy rate measurement. The main findings of this thesis are summarized and discussed in this section.

1. Hippocampal atrophy in the diagnosis of Alzheimer’s disease

Neuropathological studies have shown that hippocampal volume measured using both post-mortem and ante-mortem, MRI is associated with the severity of pathological changes in the hippocampus, which is one of the first regions to be involved in AD. Hippocampal atrophy on MRI is a well-known marker for AD, and provides support for a clinical diagnosis of AD. However, it has been suggested that the distribution of atrophy differs between early-onset (<65 years) and late-onset AD, with a relatively spared hippocampus in early-onset cases, and as such that hippocampal atrophy may be a less useful marker for differentiating between AD and controls in younger subjects. On the other hand, older age itself is associated with decreasing hippocampal volumes independent of AD. Our studies provide evidence that hippocampal atrophy is a marker for AD independent of age (chapter 3.1). We showed that the difference in hippocampal volumes between AD patients and controls was similar in a large group of young and old subjects. However, caution should be taken in subjects with an atypical non-memory presentation of AD, more commonly seen in early-onset AD, which may be associated with relatively preserved hippocampi. We cannot however estimate the effect of possible atypical AD cases on our results, since neuropsychological data were not included in this study. Further work to address this issue would however be of interest.
The utility of hippocampal atrophy in differentiating AD from other neurodegenerative diseases, such as FTLD, is less clear. AD and FTLD are two of the most common causes of early-onset dementia,\textsuperscript{12} and in clinical practice, it can be difficult to differentiate between the diseases: indeed clinico-pathological studies have described cases of FTLD presenting with severe amnesia, as well as atypical phenotypes associated with AD.\textsuperscript{11,13} MRI has the potential to be a useful tool in the differential diagnosis, although hippocampal atrophy has also been described in specific subtypes of FTLD.\textsuperscript{11,14,15} In our work, we expanded on these data by demonstrating that hippocampal atrophy is present in FTLD, as well as in AD (chapter 3.2). We observed specific patterns of hippocampal atrophy in the three subtypes of FTLD, which is consistent with the idea that the clinical phenotype of FTLD resembles the distribution of tissue loss more closely than the heterogeneous pathology underlying the disease.\textsuperscript{16,17} Hippocampal atrophy in FTLD is probably predominantly part of the overall pattern of frontotemporal atrophy, in contrast with the relatively specific and prominent early involvement of the hippocampus as seen in AD: our data suggest that hippocampal atrophy seems not to be useful as a marker in the differential diagnosis between these two diseases.

2. MRI characteristics of mild cognitive impairment

Although MCI is recognized as a risk factor for dementia, it is increasingly clear that MCI is clinically heterogeneous.\textsuperscript{18} Recent neuropathological studies have confirmed this heterogeneity, as a subset of subjects showed evidence of early Alzheimer-type pathology, often with cerebrovascular comorbidity, whereas other subjects showed different types or no neuropathology at post-mortem.\textsuperscript{19,20} The results presented in this thesis expand on these findings by providing in vivo evidence, using MRI, for the involvement of different brain substrates in MCI, associated with Alzheimer-type and cerebrovascular pathology. The degree of MTA varied considerably between individuals with MCI, and the majority of subjects had some evidence of small vessel disease (chapter 4.1 and 4.2). By investigating correlations between MRI abnormalities and cognitive measures we found that MTA was principally associated with impaired cognition. Not only was the severity of MTA related to memory deficits in keeping with previous literature,\textsuperscript{21, 22} but MTA also reflected impairment in general cognition and executive function. In parallel, MTA was associated with both amnestic and non-amnestic clinical subtypes of MCI (chapter 4.2). The association of MTA with performance in non-memory domains may reflect a more advanced disease stage, with a concomitantly more widespread atrophy, rather than a causal association with deficits in non-memory domains per se. However, the cross-sectional design of both studies prevented us from drawing conclusions on causality. Although
we demonstrated some correlation between small vessel disease, executive function and the non-amnestic MCI subtype, our results indicate a relatively modest role for small vessel disease in MCI. Our data fit in with previous longitudinal studies showing that hippocampal atrophy, rather than small vessel disease is associated with progression from MCI to AD.23–24

3. Longitudinal studies: rates of hippocampal atrophy
Several studies have reported accelerated hippocampal atrophy rates in AD. Atrophy rates in controls increase with age, but are typically about 1.0% per year, whereas in AD rates of about 4–8% per year have been described.25–27 In MCI, hippocampal atrophy rates have been reported to be intermediate between those in AD patients and controls, and to be associated with clinical progression.28–30 Thus, accelerated rates of hippocampal atrophy may reflect accumulating Alzheimer-type pathology. Previous studies have investigated risk factors associated with the development of dementia in normal or MCI subjects.30–33 We expanded on these data by identifying risk factors associated with increased rates of hippocampal atrophy, a plausible biological marker for AD, in a large cohort of MCI subjects. We identified that older age, poorer general cognition, baseline hippocampal atrophy, and the presence of an APOE e4 allele were all risk factors for accelerated rates of hippocampal atrophy in MCI (chapter 5.1). The finding that hippocampal atrophy rates are widely variable within an MCI population again emphasizes the heterogeneity of MCI. Interestingly, the vast majority of these subjects did not progress to dementia in the subsequent two years of the study, indicating that even in the earliest stages of cognitive decline accelerated hippocampal atrophy rates, similar to those observed in mild AD, can be detected and that these subjects share well-reported risk factors for AD.

Whilst manual segmentation is currently considered the gold standard technique for measuring hippocampal volumes, errors in delineation easily occur as the hippocampus is an anatomically complicated structure. New methods of hippocampal volume measurement are evaluated to improve both efficiency and reliability. Newly proposed automated measures are promising, but still require manual intervention, such as the selection of landmarks, and are not yet fully validated.34–36 An alternative, semi-automated method based on non-linear registration of serial MRI scans has been described, and previous work has shown this to be reliable in differentiating AD patients from controls on the basis of hippocampal atrophy rates.37 We further assessed this new method (chapter 5.2) and showed, besides good agreement with manual measurement, improved reliability for hippocampal atrophy rate measurement compared to the gold standard. Hippocampal atrophy rates have already been used as surrogate outcome measures in medication trials in AD and MCI.38–40 Future
trials, assessing large numbers of subjects, could benefit from improved reliability and efficiency of the local fluid registration. It would be expected that smaller sample sizes would be sufficient to demonstrate possible medication effects if measurement error is reduced using this technique. However, in our study, we could not demonstrate a reduced variability in hippocampal atrophy rates as derived by local fluid registration compared to manual measurement, which may be explained by true variability within subjects in a relatively small study. Future studies applying local fluid registration to larger cohorts are needed.

**Implications**

The results presented in this thesis expand on the use of hippocampal atrophy on MRI in the diagnosis of AD. We have shown that hippocampal atrophy is a marker for early-, as well as late-onset AD, provided that the severity of hippocampal atrophy is evaluated in comparison to age-matched controls, and we recommend the use of age-specific cut-off values for hippocampal atrophy in the diagnosis of AD. Caution should be taken using hippocampal atrophy as a marker for AD in the differential diagnosis with other types of dementia. Our results show that hippocampal atrophy on MRI is not specific for AD, and that a diagnosis of FTLD is not excluded by hippocampal atrophy on MRI. However, severe asymmetrical left-sided hippocampal atrophy may point to a diagnosis of semantic dementia.

Our results also indicate that hippocampal atrophy is not only important in the diagnosis of AD, but can contribute to a better understanding of predementia stages. Currently, it is not possible to select accurately subjects at risk for AD. Although subjects meeting criteria for MCI are at an increased risk for AD, MCI is a clinically and pathologically heterogeneous concept. This thesis shows that the heterogeneity of MCI is reflected in a wide range of severity of MRI abnormalities, including MTA and cerebrovascular pathology. MTA proved to be the strongest predictor of cognitive deficits. We suggest incorporating the presence of MTA in the selection of subjects at risk for AD for future studies or enrichment of clinical trial cohorts. The recently developed revised research diagnostic criteria for probable AD require one or more abnormal biomarkers to be present, including evidence of MTA assessed on structural MRI, in addition to episodic memory impairment. However, it is stressed that non-AD disorders associated with MTA should be ruled out using careful clinical evaluation.

The prediction of future cognitive status at an individual level using cross-sectional measures of hippocampal atrophy is limited by the restricted sensitivity and the large
inter-individual variation in brain morphology. Longitudinal measures of hippocampal volume change over time may be more sensitive to discover the early changes associated with AD. Demonstration of accelerated hippocampal atrophy rates could provide prognostic information to individual patients in early stages, and could be used to include more homogeneous patient groups in clinical trials. We identified the following factors to be associated with increased hippocampal atrophy rates in MCI: older age, poorer general cognition, pre-existing hippocampal atrophy and the presence of an APOE e4 allele. These factors may aid identification of subjects at risk for developing AD. Finally, the semi-automatic local fluid method seems to be a promising method for hippocampal atrophy rate measurements on serial MRI.

**Recommendations for further research**

*Risk factors for and course of hippocampal atrophy rates*

The focus in the use of MRI in dementia research is shifting from cross-sectional measures towards longitudinal assessment of structural brain change. Serial MRI scanning offers the potential to detect the earliest brain changes associated with neurodegenerative disease, and to monitor the progression of the disease. In this thesis we identified predictors of accelerated hippocampal atrophy rates in MCI, more severe hippocampal atrophy and poorer general cognition proving to be two of those factors. However, the ultimate goal would be to identify subjects at risk for AD before irreversible, neuronal damage, associated with hippocampal volume loss and cognitive deficits, have taken place. Little is known about the onset and course of atrophy of the hippocampus and other brain structures in very early, and presymptomatic disease stages. Familial AD forms an ideal opportunity to study the earliest brain changes associated with AD. Very interesting in this respect is a recent study by Ridha et al demonstrating that accelerated rates of hippocampal and global brain atrophy can differentiate between mutation carriers and controls as early as 5.5 and 3.5 years respectively before diagnosis of (familial) AD. Thus far only a few promising studies exist assessing the course of brain atrophy in cognitively unimpaired controls that progress to sporadic AD during follow-up, with generally small sample sizes and limited length of follow-up. Larger, prospective, studies are needed to assess measures of brain atrophy over time on MRI, in relation to development of cognitive decline and incident dementia. Ideally, a large, epidemiological study following subjects without cognitive symptoms should be conducted. Alternatively, MCI subjects could be recruited, and compared to subjects with AD and cognitively unimpaired controls. Subjects should be followed for a long period of 5-10 years (or longer) and preferably have more than two MRI scans. MRI measures of interest
could consist of cross-sectional volume and longitudinal volume change of different brain structures such as the hippocampus, entorhinal cortex, ventricle size and global brain volume. In addition, measures of small vessel disease could be used to assess possible interactive or additive effects of this commonly observed pathology. Such a study would allow assessment of the onset and course of brain atrophy, and the temporal relationship between various MRI measures. Predictive values of cross-sectional and longitudinal MRI measures for cognitive decline and incident dementia could be calculated. Finally, such a study would allow the identification of possible genetical or environmental risk factors associated with brain changes. This could improve our knowledge regarding the pathogenesis, and progression of AD. For clinical practice, the identification of modifiable risk factors would be of particular relevance.

Combining MRI measures with cerebrospinal fluid markers

Besides structural MRI measures, several other biomarkers for AD are being investigated. Combining different markers may improve understanding of the processes underlying abnormalities that are detected with specific markers and increase diagnostic accuracy. The cerebrospinal fluid (CSF) markers amyloid \( \beta \) 1-42, tau and phosphorylated tau (Ptau) are assumed to reflect the neuropathological processes in AD. In CSF a combination of decreased concentrations of amyloid \( \beta \) 1-42 and elevated concentrations of tau and/or Ptau are able to differentiate AD patients from control subjects with high accuracy. Two recent studies combining MTA and CSF markers have found an additive value of both markers in the differentiation between patients with AD and controls, and the prediction of AD in subjects with MCI. CSF markers may predict AD earlier in the disease course, whereas the presence of MTA could predict progression over a shorter interval. Studies combining longitudinal MRI data and CSF are scarce, two interesting small studies have shown that increasing baseline levels of P-tau are associated with accelerated rates of hippocampal atrophy in subjects with AD and MCI. Another possible advantage of the use of CSF biomarkers is their assumed specificity for AD, whereas structural MRI can visualize hippocampal atrophy in great detail but, as we demonstrated, does not differentiate between possible underlying pathological processes. However, in contrast with longitudinal measures of atrophy on MRI that are assumed to track disease progression, the value of longitudinal CSF analysis is debated, and is thought to be less useful to detect disease progression. Adding CSF analysis, at least at baseline but preferably at each MRI time-point, to the study outlined above, would allow comparison of predictive values of CSF and MRI measures for future cognitive decline, their temporal relation, and associations with other clinical variables.
REFERENCES


