SUMMARY AND GENERAL DISCUSSION
The overall objective of this thesis was to provide insight in cognitive profiles and rate of cognitive decline in AD and to explore which factors are involved in driving the different phenotypes. In the previous chapters these studies were described in detail, focusing on the role of a) APOE genotype, b) CSF biomarkers and c) MRI measures in relation to 1) profiles of neuropsychological impairment, and 2) rate of cognitive decline. In this chapter, the main findings are summarized and relevant issues related to the studies are discussed. The chapter closes with clinical implications of our findings and suggestions for future research.

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<th>Summary and general discussion</th>
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Profiles of neuropsychological impairment in AD

The APOE ε4 genotype increases the risk of AD and has been associated with an earlier age at onset. In chapter 2.1, we examined whether impairment in specific cognitive domains in AD differed according to APOE genotype and age at onset. Cognitive functions of 229 consecutive AD patients were assessed using a short neuropsychological test battery. APOE ε4 carriers showed more memory impairment than APOE ε4 non-carriers, while in contrast, APOE non-carriers were more severely impaired in naming, mental speed and executive functions. Some of these associations were modified by age at onset, although no main effect for age at onset was found. The APOE effect on memory was most pronounced in early onset homozygous carriers. With regard to naming, older patients performed better as their number of APOE ε4 alleles increased, while younger patients performed worse with increasing number of APOE ε4 alleles. The APOE genotype seems to influence not only the age at onset in sporadic AD patients, but also the cognitive phenotype once patients have reached the stage of dementia.

Chapter 2.2 investigated the relationship between CSF biomarkers and cognitive profiles in AD. We included 177 AD patients who underwent a short neuropsychological assessment. In CSF, levels of Aβ1-42, tau and p-tau were measured. K-means cluster analysis was performed with the three biomarkers to obtain 3 clusters of patients. Cluster 1 consisted of 88 patients with relatively high levels of Aβ1-42 and low levels of tau and p-tau. Cluster 2 contained 72 patients with relatively low levels of Aβ1-42 and high levels of tau and p-tau. Cluster 3 was made up of 17 patients with low levels of Aβ1-42 and very high levels of tau and p-tau. No differences between clusters on age, sex, education, APOE genotype, disease duration, functional impairment or disease severity were found. We found that a subgroup of patients with extremely high CSF levels of tau and p-tau showed a distinct cognitive profile with more severe impairment of memory, mental speed and executive functions, which could not be explained by disease severity. In contrast, patients with levels of Aβ1-42, tau, and p-tau close to normal showed less impairment of naming and memory abilities than patients with more extreme biomarker levels.

In Chapter 2.3 we aimed to assess the associations of global atrophy and WMH with neuropsychological function in early and late onset AD. We included 107 patients with sporadic AD (21 early onset and 86 late onset) from our memory clinic. Tests for (working)
memory, language, executive functions, mental speed and attention were administered. Global atrophy and global and lobar WMH were measured using MRI. Linear regression analyses showed that global atrophy was associated with more severely impaired global cognition, working memory, mental speed and executive functions. Significant interactions between global atrophy and age at onset showed that these associations were mostly attributable to patients with early onset AD. By contrast, an association between global atrophy and memory was found, which was specifically attributable to late onset AD patients. No associations between global WMH and cognitive function were found, but analyses of regional WMH showed that temporal WMH was associated with impaired memory, and frontal WMH with slower mental speed. We concluded that cortical atrophy, a key feature of AD, is linked to a wide range of cognitive functions, specifically in early onset AD patients. WMH was not influenced by age at onset, indicating that, if WMH are present in early onset patients, they have a similar effect on cognition as in older patients.

Rate of cognitive decline in AD

Our hypothesis that APOE genotype is involved with more than the risk of developing AD and the age at onset alone was supported by our findings in chapter 3.1. In this longitudinal study, we aimed to compare the rate of cognitive decline in patients with early and late onset AD and to investigate the potentially modifying influence of APOE genotype. We included 99 patients with early onset AD and 192 patients with late onset AD who had at least 2 MMSE scores (range 2-14) obtained at least one year apart. Linear mixed models were performed to investigate the rate of cognitive decline dependent on age at onset and APOE genotype. Age at onset was not associated with baseline MMSE. However, with 2.4 points decline per year, patients with early onset showed faster decline than patients with late onset (-1.7 points/year). After stratification according to APOE genotype, APOE ε4 non-carriers with early onset showed faster cognitive decline than non-carriers with late onset (-2.4 vs -1.3 points/year). In APOE ε4 carriers, no difference in rate of cognitive decline was found between patients with early and late onset. We concluded that patients with early onset AD show more rapid cognitive decline than patients with late onset, suggesting that early onset AD follows a more aggressive course. This effect seems to be most prominent in patients with early onset that do not carry the genetic APOE ε4 risk factor for AD.

In chapter 3.2, the relationship between CSF biomarkers and cognitive decline was studied using a longitudinal design. We hypothesized that high tau, especially in combination with relatively low p-tau, is a marker of rapid decline, since it has been associated with fast neuronal degeneration. We included 151 AD patients, of whom we had baseline CSF, from our memory clinic. All patients had at least two MMSE scores, obtained no less than one year apart. Mean follow-up period was 2 years. Linear mixed models were used to assess associations between CSF biomarkers and the rate of cognitive decline as measured with the MMSE. No relations between any CSF biomarker and baseline MMSE were found. However, CSF biomarkers did predict cognitive decline over time. A low p-tau/tau-ratio
was the strongest predictor with a dose dependent effect (lowest vs highest quintile: 2.9 vs 1.3 MMSE-points annual decline), indicating that a combination of high CSF tau without proportionally elevated p-tau is associated with a faster rate of cognitive decline. In addition, low Aβ_{1-42}, high tau and high tau/ Aβ_{1-42}-ratio were also associated with rapid cognitive decline.

In chapter 3.3 we investigated the relationship between brain microbleeds and the rate of cognitive decline in AD. We studied 221 AD patients with available baseline MRI (1.0 or 1.5T) and at least two MMSE scores obtained at least one year apart from our memory clinic. Mean follow-up duration was 3 years and patients had a median of 4 MMSEs. There were 39 patients with microbleeds (median = 2, range 1-27) and 182 without. Linear mixed models showed that overall, patients declined 2 MMSE points per year. We found no relation between the presence of microbleeds and baseline MMSE or change in MMSE over time. Adjustment for atrophy, WMH, lacunes and vascular risk factors did not change the results, nor did stratification according to microbleed location, APOE ε4 carriership or age at onset (≤65 years vs >65 years). Repeating the analyses with number of microbleeds as predictor rendered similar results. We concluded that microbleeds do not influence the rate of cognitive decline in AD patients. The formerly reported increased risk of mortality in patients with microbleeds seems not to be attributable to a steeper rate of decline per se, but might be due to vascular events, including (hemorrhagic) stroke.

**METHODOLOGICAL ISSUES**

**Selection of patient population**

This thesis has a unique approach to studying cognition in AD. Rather than comparing AD patients to healthy controls, we have studied the cognitive spectrum within the disease itself, acknowledging that not all AD patients are the same, but that cognitive, biological and pathophysiological factors vary greatly amongst AD patients. Studying risk factors such as age at onset and APOE ε4 genotype in relation to variability in clinical manifestations of AD, as opposed to comparing AD patients to healthy elderly, has lead to increased understanding of the biological factors underlying this phenotypical heterogeneity.

Since the diagnosis of early onset dementia is a point of great interest to the VUmc Alzheimer Center, the group of early onset AD patients is overrepresented in the cohort, offering unique potential: younger AD patients suffer from a more pure form of the disease, which makes them an ideal model to study the pathogenesis of AD. Furthermore, heterogeneity in manifestation is especially evident within the group of early onset AD.

Several factors may have led to a biased selection of patients for our studies. With regard to the studies of neuropsychological profiles in AD, in two studies (Chapters 2.2 and 2.3) we have only used patients with complete neuropsychological data. Since patients that are more severely cognitively impaired are often unable to complete all neuropsychological tests and
therefore have missing data on one or more tests, this may have led to a bias in our results. We cannot rule out that these patients may have a different cognitive profile than patients who were able to complete all tests, although we have no indication that more severely cognitively impaired patients would have a distinctly different cognitive profile. Rather, as patients become more severely demented, more cognitive domains become impaired, making it harder to distinguish cognitive profiles and even to differentiate between the various types of dementia. Therefore, it seems to be a strength that we have only included patients who were mildly demented.

The studies of rate of cognitive decline may have been subject to survivor bias: patients with extremely fast decline, severe disability or short survival, which prevents them from returning to the memory clinic, may have been lost to follow-up. This may have resulted in a possible underestimation of the rate of decline of all or some of the reported subgroups. This is especially relevant for the microbleeds study, in which we found no effect on cognitive decline, while previous cross-sectional studies have reported a relationship between microbleeds and cognition, and between microbleeds and risk of mortality. However, it should be noted that there are other, cross-sectional, studies that also found no relationship between microbleeds and cognition.

**Neuropsychological assessment**

The studies in Chapter 2 are based on a standard test battery that screens several cognitive domains. This gives more detailed information than the use of cognitive screening tests such as the MMSE or the CAMCOG alone. Since this battery is used for every patient in our memory clinic, we have been able to study specific cognitive domains in large patient samples, adding to the reliability of the results.

The test battery lacks a cognitive test specifically designed to assess praxis and visuospatial functions. An adequate measure for praxis and visuospatial functions is hard to find and therefore most studies do not include such a test. This is particularly relevant for AD studies, since often these domains are among the first to be impaired in non-amnestic AD patients, while by contrast they may be preserved for a relatively long time in typical, amnestic AD patients. It would therefore be advisable to include tests for praxis and visuospatial functions in the standard test battery. In the last few years, three subtests of the Visual Object and Space Perception Battery (VOSP) have been added to the test battery to assess visuospatial functions. In a recent study, our lab showed that patients with early onset AD performed worse on several non-memory domains, amongst which visuospatial functions, than late onset AD patients. Lately, van Heugten's test for apraxia has been added to the test battery and data on praxis abilities in our cohort are now being collected.

For the studies in Chapter 3, we have used the MMSE as a measure for cognitive decline. Although the MMSE is only a short cognitive screening test and a rather crude cognitive measure, it is a generally accepted - and in our experience a robust - measure for cognitive
A large advantage of the MMSE is that it is easy to administer, which has enabled us to collect data from a large number of patients over a long period of time, even measured at up to 17 time points. A possible disadvantage is that the MMSE only allows us to study global cognitive decline, whereas it would also be interesting to study decline in different cognitive domains. Since a few years, we have started to subject patients to a neuropsychological assessment at multiple time points. However, we need to be aware that as this is more straining on the patients, the risk of survivor bias will increase. Therefore, studies of global cognitive decline as reported in this thesis, together with studies of decline in specific cognitive domains that are currently going on, will give a more complete picture of the mechanisms underlying decline in AD.

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<th>CLINICAL IMPLICATIONS</th>
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<td>Among the general public, but also among primary care physicians, a stereotypical picture of AD still exists. It is the classical picture of an older person with predominantly memory problems, which slowly get worse, followed by the impairment of other cognitive functions, until finally the patient can no longer take care of him/herself, is admitted to a nursing home and dies. This thesis has shown that this general idea should be adjusted. Many AD patients suffer from impairment in other cognitive domains, such as language, mental speed and/or executive functions, while their memory function is relatively spared in the early stages.</td>
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We were able to link genetic, imaging and pathophysiological substrates to cognitive subtypes of AD. These findings may have important clinical implications. In recent years, more and more effort has been directed towards the development of therapies for AD. The heterogeneous nature of AD suggests that it is not very likely that a single therapy (“magic bullet”) will benefit all AD patients. In a future of “personalized medicine”, it is increasingly important to be able to identify meaningful subtypes, which all will need a different approach. Our results suggest that variability in clinical manifestation in AD is related to biological substrates, which may point at specific pathways. It is conceivable that combining cognitive profile and biological markers will be helpful in the tailor-made allotment of future therapies.

Many drug trials use cognitive change as their primary outcome measure in testing the efficacy of the drug in question. This thesis has shown that age at onset, APOE genotype, and CSF biomarkers are all related to the rate of cognitive decline in AD. It is advisable for designers of drug trials to take these factors into account when evaluating new interventions. As one example, the interest in the clinical consequences of microbleeds has recently grown, since amyloid related imaging abnormalities (ARIA) including hemosiderin deposition (ARIA-H) have occurred in patients participating in clinical trials with therapeutic agents to lower amyloid-β burden in AD. In this context, our finding of a lack of association between microbleeds (as one form of ARIA-H) and the rate of cognitive decline may be of importance. If the rate of cognitive decline is not influenced by the presence and number
of microbleeds, excluding patients with microbleeds may not be necessary for that reason. However, it should be noted that the prognosis of ARIA-H may be different from that of spontaneously occurring microbleeds. Therefore, further research is needed regarding the risk of accelerated cognitive decline in patients with ARIA-H.

| RECOMMENDATIONS FOR FUTURE RESEARCH |

In the literature, several attempts to define subtypes in different modalities (e.g. cognition, MRI, CSF) have been made. In this thesis, we have categorized patients based on biological and pathophysiological factors and have studied whether this resulted in distinct neuropsychological profiles. The next step is to look at the data from the opposite direction, by first determining which cognitive clusters of AD patients can be found. Clustering of cognitive data should be done in a data driven manner, without a priori hypotheses with regard to the number or type of subpopulations, and the results should be validated in another sample to test their generalizability. Subsequently, using a more holistic approach, efforts should be made to relate the subtypes to the combination of biological and pathophysiological factors such as APOE genotype, CSF biomarkers, EEG abnormalities, MRI-measures and age at onset.

Furthermore, besides studying the relationships between cognitive phenotypes and known biological and pathophysiological factors, major efforts should be put in the quest for new biomarkers in blood, CSF or other fluids. Biomarkers are needed to predict disease progression (prognostic markers) and to predict response to therapy (theragnostic markers). Biological factors, which show meaningful variation within the spectrum of AD, are likely candidates for these purposes. For example, it is generally accepted that other genetic factors beside APOE genotype influence the pathogenesis of sporadic AD. Genomewide association studies to date have chiefly focused on identifying disease susceptibility factors. The next step is -in stead of comparing the DNA of AD patients to the DNA of controls- to relate the DNA of different cognitive subtypes of AD with each other in an endeavour to identify disease modifying factors.

Following on the idea of integrating data on cognitive subtypes with biological, genetic and pathophysiological factors in a holistic approach, this could be extended to the rate of cognitive decline. Following up on the studies in this thesis that have looked at the relationship between individual factors such as APOE genotype, CSF biomarkers and age at onset, it would be interesting to develop an algorithm based on these and other biological, genetic and pathophysiological factors that can predict the rate of cognitive decline of individual AD patients.

Machine learning algorithms have recently been proposed to combine multiple AD features derived from brain imaging and other biomarkers, for Mild Cognitive Impairment (MCI) and AD classification and for predicting conversion from MCI to AD. Several studies have performed diagnostic classification based on several MRI measures. Others have
extended on this by adding data from other modalities, such as incorporating demographic
variables such as age, sex, and APOE genotype and including CSF biomarkers, and PET
data. The next step would be to combine data of different modalities in order to predict
the rate of cognitive decline once the patient has developed dementia. Besides the obvious
benefit for the patient and his/her caretakers of being able to predict the rate of cognitive
decline, it may provide vital information for designing, powering, and implementing future
clinical trials.


