SUMMARY OF MAIN FINDINGS

Understanding cognitive heterogeneity in AD

In chapter 2 we aimed to better understand cognitive heterogeneity in AD. Previous studies had already described distinct cognitive AD subtypes such as the logopenic progressive aphasia, the dysexecutive/behavioural subtype, and posterior cortical atrophy.\textsuperscript{1-4} Cognitive heterogeneity in AD is however not restricted to these extreme variants, but also present in the more typical spectrum of AD. We therefore aimed to capture cognitive heterogeneity using cluster analyses of neuropsychological test results in AD dementia patients to identify cognitive AD phenotypes. We compared identified phenotypes in terms of neurobiological characteristics, and in terms of disease progression over time, investigated using repeated cognitive screening tests and mortality data.

For our first clustering study in paragraph 2.1 we included a large sample of AD dementia patients from the Amsterdam Dementia Cohort, and performed latent class analyses (LCA) of neuropsychological test results collected from an extensive neuropsychological test battery. The clustering solution with eight subtypes fitted the data best. Subtypes were characterized by distinct cognitive profiles. Two clusters, including 43% of patients, were characterized by most prominent memory impairment (the memory phenotype). Three clusters, including 29% of patients, were characterized by most prominent non-memory impairment (visuospatial functioning, language, and/or executive functioning) and relative sparing of memory (the non-memory phenotype). The other three clusters, including 28% of patients, were characterized by a cognitive profile without prominent impairment or sparing of memory (the memory indifferent phenotype). The non-memory phenotype was associated with younger age, apolipoprotein (APOE) e4 negative genotype, and relative sparing of hippocampal atrophy. We concluded that this cluster technique was able to identify cognitive AD phenotypes in the Amsterdam Dementia Cohort, associated with distinct neurobiological characteristics. Since this cohort includes patients recruited from the outpatient memory clinic of Alzheimer Center Amsterdam, specialized in early-onset dementia, we acknowledged hampered generalizability of findings for the worldwide AD dementia population. We therefore aimed to increase
generalizability and to validate findings based on several cohorts from over the world in the next paragraph.

In **paragraph 2.2** we included four large and independent AD dementia cohorts from Europe and the USA. Cohorts differed in patient populations and exact composition of neuropsychological test batteries. For this validation cluster analysis study, we used nonnegative matrix factorization (NMF) of neuropsychological test results. Cluster solutions fitted the data best when patients were clustered into two subtypes in all four cohorts. Clusters were robustly characterized by most prominent memory impairment (the memory phenotype), or by relative memory sparing (the non-memory phenotype). In line with findings of paragraph 2.1, the non-memory phenotype was younger, more often APOE e4 negative, and had relative sparing of the hippocampus but more severe posterior atrophy compared with the memory phenotype. We confirmed the conclusion that cognitive heterogeneity could be captured by clustering neuropsychological test results of AD patients, and that identified phenotypes are associated with distinct neurobiological characteristics. In addition, in both paragraphs 2.1 and 2.2, the non-memory phenotype was associated with shorter duration of complaints and lower (worse) Mini-Mental State Examination (MMSE) scores at diagnosis. We hypothesized that this phenotype suffered from faster disease progression, which we further investigated in paragraph 2.3.

In **paragraph 2.3** we elaborated on previous findings and performed a longitudinal study to investigate disease progression in cognitive phenotypes identified in paragraph 2.2. We explored disease progression using repeated MMSE scores, Clinical Dementia Rating scale sum of boxes (CDR sob), and mortality. Using linear mixed models, we found that the non-memory subtype was associated with faster disease progression (steeper decline on MMSE scores and increase on CDR sob). Furthermore, we performed Cox proportional hazard analysis in one cohort where mortality data were available and found that the non-memory subtype was associated with higher mortality rate.

Main conclusions of chapter 2 were that AD is a heterogeneous disease, and that cognitive heterogeneity could be reliably captured using cluster analysis of neuropsychological test results. Furthermore, identified cognitive phenotypes were associated with distinct neurobiological characteristics and different rates of disease progression and mortality.
Applications of $^{18}$F-FDG-PET in AD

$^{18}$F-FDG-PET could be of great importance when differentiating AD from other causes of dementia. In chapter 3 we aimed to assess $^{18}$F-FDG-PET in AD visualising cerebral glucose metabolism as a diagnostic marker, and as an outcome marker for clinical trials.

In paragraph 3.1 we investigated the specificity of posterior cingulate cortex (PCC) hypometabolism in AD. PCC hypometabolism is usually associated with AD\textsuperscript{5,6} and could therefore be helpful to differentiate between AD and other causes of dementia. Based on our own clinical expertise however, PCC hypometabolism could be present in other causes of dementia as well, e.g. the behavioural variant of frontotemporal dementia (bvFTD). We investigated presence of PCC hypometabolism in AD, bvFTD, and cognitively normal subjects based on visual reading of $^{18}$F-FDG-PET scans. We then validated presence of PCC hypometabolism in bvFTD using PCC $^{18}$F-FDG standard uptake value ratios, with a cut-off for hypometabolism, derived based on the receiver operating characteristic separating AD from cognitive normal subjects. We found that PCC hypometabolism is not restricted to AD and could be present in almost a third of bvFTD patients as well. Lacking specificity for AD, we therefore concluded that PCC hypometabolism as solitary biomarker to differentiate AD from other neurodegenerative diseases is not sufficient. Visual interpretation of the total pattern of cerebral glucose metabolism, or using a specific AD template, is required, and a combination with additional biomarkers (e.g. amyloid biomarkers) should be considered.

In paragraph 3.2 we investigated $^{18}$F FDG-PET as an outcome measure for clinical trials. Based on a literature study, $^{18}$F-FDG can be considered a direct index for synapse function and density. We hypothesised applicability of $^{18}$F-FDG-PET to explore the effect of nutritional intervention (i.e. Souvenaid\textsuperscript{®}) – designed to improve synapse function and formation – over time in a randomized clinical trial in a population of AD patients. We designed the NL-ENIGMA study; a Dutch study exploring the Effect of a Nutritional Intervention on cerebral Glucose Metabolism in early AD. No previous studies have investigated the effect of a nutritional intervention on cerebral glucose metabolism. We defined early AD as mild cognitive impairment (MCI) or dementia with MMSE score $\geq$ 20, and AD cerebrospinal fluid
or PET biomarker results suggestive for underlying AD pathology. Main outcome parameters included the effect of the nutritional intervention on change in glucose metabolism as measured with quantitative and semi-quantitative $[^{18}\text{F}]$FDG-PET imaging at baseline and after 24 weeks intervention (test product or placebo).

In paragraph 3.3 we presented main results of the NL-ENIGMA study. We included 50 early AD patients, each treatment arm (test product or placebo) consisted of 25 randomly allocated patients. Per protocol populations included 42 patients based on availability of semi-quantitative measures (control $n=20$, intervention $n=22$), and 37 patients based on availability of quantitative measures (control $n=19$, intervention $n=18$). Treatment arms were comparable in terms of baseline characteristics, including semi-quantitative and quantitative $[^{18}\text{F}]$FDG-PET measures. Based on region-of-interest analyses as well as voxel-based analyses, no differences between treatment arms were found in terms of change in glucose metabolism over time. Noticeable is that the treatment arm did not show increase in metabolism, nor did the placebo arm show decrease in metabolism over time. As such we were not able to not draw strong conclusions regarding applicability of $[^{18}\text{F}]$FDG-PET to capture change in synapse function over time, since this study was most likely hampered by the short follow-up duration. This study provides perspectives for future research though, as described later in this chapter.