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Secondary Prevention for Alzheimer Disease

Timing, Selection, and Endpoint of Clinical Trials

Lisa Vermunt

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Secondary Prevention for Alzheimer Disease

Timing, Selection, and Endpoint of Clinical Trials

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Chapter 1

General introduction

Alzheimer disease (AD) is the most common cause of dementia, accounting for 50-70% of the estimated 46 million patients with dementia world-wide [1, 2]. AD dementia is a main cause of disability and death, and has a major impact on the lives of patients and their families [3, 4]. The disease is defined by amyloid plaque and tau tangle formation in the brain, which are accompanied by neurodegeneration and cognitive decline [5]. Dementia is the end-stage of AD [6]. There is currently no treatment to slow or halt the disease. Despite significant investments of pharmaceutical companies, investigators, study participants and their caregivers, all clinical trials thus far have failed [3]. The simple explanation for the negative results would be that all treatment compounds were ineffective. Still, in retrospect, there can also have been shortcomings in the design of the trials, in particular the participant selection and timing of the interventions [7].

1.1 Clinical trials in relation to biomarker developments

One major issue hampering clinical trials in AD in the past was diagnostic uncertainty. According to screening data of previous clinical trials 10-25% of patients with a clinical diagnosis of AD-type dementia did not have evidence amyloid plaque accumulation in the brain [8-10]. This is particularly problematic for experimental treatment studies, because many of the compounds target amyloid plaques [11]. During the past two decades, biomarkers became available that can measure amyloid accumulation during life using cerebrospinal fluid (CSF) or positron emission tomography (PET) imaging. The use of these biomarkers allows confirmation of AD pathophysiology in patients with AD dementia at study enrolment, which ensures that the right patients are treated.

Using AD biomarkers, it became clear that AD pathology may be present long before the onset of dementia [12, 13]. Individuals with amyloid pathology may be treated during this period to delay or prevent the onset of dementia [14-16]. Another explanation for the lack of treatment effects is that the interventions were initiated too late in the disease process. An hypothesis is that equivalent interventions may be effective when started earlier, e.g., in pre-dementia AD [17]. In a new branch of research into pre-dementia AD, individuals without dementia undergo AD biomarker measurements and are followed over time. To conduct trials in pre-dementia AD, we need to understand when to intervene, how to find suitable participants, and develop methods to evaluate effectiveness in pre-dementia AD. Those are topics investigated in this thesis.

This chapter has the following structure: (2) a brief summary of the current hypothesis on the development of AD and explanation of relevant terminology and methods, which both provide background for the following chapters, (3) progress and challenges in clinical trials for AD, (4) project descriptions, (5) the specific aims and outline of this thesis.

2 Understanding and defining Alzheimer disease

2.1 Biological progression model of Alzheimer disease

In 1992, Hardy and Higgins pose the amyloid cascade hypothesis [18], which Jack and colleagues adapt into the disease progression model of AD, based on early biomarker studies [19]. According to this hypothesis, AD dementia develops in a sequential order of biomarker and clinical abnormality over decades. The first sign is amyloid accumulation, followed by neuronal injury and dysfunction, neurodegeneration, cognitive decline, and functional decline (Figure 1). Several publications support this AD progression model. Firstly, early biomarker studies show that 30% of

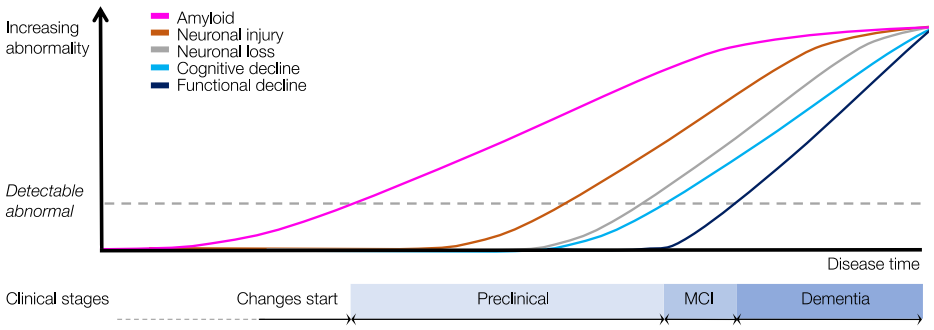

Figure 1 Alzheimer disease progression model and clinical stages
Adapted from Jack et al. 2013 [5].

individuals, who are older than 65 years have evidence of amyloid accumulation in their brain, but have no dementia [20], which is also conform neuropathology studies. It indicates that pathophysiological changes start before symptoms are present [5]. Second, there is a gap of ~20 to 30 years between the increases of amyloid accumulation and AD-type dementia prevalence [20]. Based on amyloid accumulation rates, the pre-dementia period is estimated to be approximately 17 years [21]. Lastly, in the presence of AD pathology, cognitively normal individuals show higher progression rates to mild cognitive impairment and dementia compared to individuals without AD pathology [22-25]. This evidence suggests that there is a long pre-dementia period as window of opportunity for interventions to prevent dementia, warranting further investigation.

2.2 Clinical stages of Alzheimer disease

To study pre-dementia stages of AD, research expert groups developed criteria which divide AD into clinical stages [14, 26, 27]. These criteria have been updated several times over the past 10 years. In this thesis, I use an amyloid-centric definition: if amyloid accumulation is present, this is referred to as AD. Preclinical AD refers then to individuals without any signs of cognitive impairment. Prodromal AD and mild cognitive impairment (MCI) due to AD are both referring to the mild cognitive impairment stage, in which there is cognitive impairment, but no functional impairment. In AD dementia, patients have become dependent on others in their activities of daily living, as a result of progressive cognitive impairment [6]. AD dementia has a mild, moderate and severe dementia stage, according the level of functional dependence on others.

Clinically, most individuals progress from normal cognition via mild cognitive impairment to dementia, but the duration of the stages has not been well-described (see Ch. 2.1 of this thesis). Some individuals revert to less severe stages or fluctuate between clinical stages [28]. This clinically deviating group of patients are interesting to study as they might inform us on the prognostic factors for clinical progression of AD (Ch. 2.2).

2.3 Risk factors for Alzheimer disease

Many risk and protective factors for AD dementia have been identified, including genetic and environmental factors [2]. In less than 1% of patients, AD is caused by a genetic mutation in the PSEN1, PSEN2 or APP gene. The most common genetic risk factor for AD dementia is the presence of one or two *APOE ε4* alleles. There are also many risk and protective factors found in epidemiological studies of which the mechanisms are unknown. Risk factors for AD type-dementia include female sex, lower level of education, hypertension, and depressive symptoms. More exercise and more social and intellectual engagement seem protective. The effect of these risk factors can also be stage-specific (Ch. 2.1) Unraveling risk factors for AD dementia can help to target treatments or stratify clinical trial enrolment and evaluation of outcomes (Ch. 3.2).

2.4 Structural neuroimaging and connectivity

In AD, patients have neurodegenerative changes that can be detected and characterized with structural brain imaging. For clinical trials, structural magnetic resonance imaging (MRI) can be useful for selecting participants most likely to undergo cognitive decline, or to measure treatment response [29]. The temporal cortex appears preferentially vulnerable to atrophy in AD. Well-established techniques for assessing temporal lobe atrophy include visual medial temporal lobe atrophy (MTA) rating scale and automated volumetrics [30-32]. More recent studies are able to measure cortical thickness in multiple brain regions, suggesting that cortical thinning in multiple brain regions, including in the parietal lobe, may be a sensitive marker of AD-related changes [33]. Growing evidence also suggests disruptions in grey matter connectivity as an early feature of AD [34-36].

In this thesis, we apply the single-subject whole-brain grey matter covariance network approach (Ch. 4) [37]. This method is based on the fact that brain structures develop and maintain in an organized manner, which results in similarity between brain areas and that is correlated to healthy brain function [38, 39]. This similarity can be described as a network using graph theory properties, such as the number of nodes and connections, the average path length between nodes and the level of clustering (see Box 1 Chapter 4.1 on page 116 for details). The networks have previously been shown to be disrupted in AD dementia patients [36]. Additionally, in cognitively healthy individuals grey matter network disruptions are associated with amyloid accumulation levels [40, 41]. This suggests that network changes occur early in the disease and that this may be developed into an endpoint for clinical trials in pre-dementia AD. Studying brain connectivity can also be useful to better understand the development of the disease.

2.5 Cerebrospinal fluid biomarkers

CSF protein levels are used to diagnose AD, as well as to study biological changes (Ch. 4.2). The proteins used for diagnosis AD include reflections of β-amyloid (Aβ) and tau (phosphorylated [pTau], total [tTau]) accumulation. More biological processes can be reflected in the CSF by protein levels, such as amyloid processing, neurodegeneration, inflammation and synaptic damage [42-47]. We use the ratio of $A\beta_{42/40}$ as a marker of amyloid aggregation, $A\beta_{40}$ for amyloid processing, pTau for hyperphosphorylation of tau and tTau for neuronal injury. Neuronal calcium-sensor protein (VILIP1) reflects neuronal death and neurofilament light chain (NfL) axonal degeneration. Furthermore, levels of chitinase-3-like protein 1 (YKL-40), an astrocyte marker, and soluble TREM2, a microglia marker, are assessed to detect inflammation. SNAP-25 is used to detect presynaptic damage and neurogranin (Ng) to detect postsynaptic damage. Combining CSF markers with grey matter connectivity may allow delineation of which processes contribute to network disruptions over the AD trajectory (Ch. 4.2).

3 Clinical trials

3.1 Clinical trials for prevention of AD

Three types of prevention exist in medicine. The first type is primary prevention, referring to a preventive treatment for individuals without pathological signs of the disease. In AD, one testable hypothesis can be to prevent amyloid accumulation by intervening in the amyloid production. Secondary prevention applies to individuals with pathological signs of the disease, who do not yet exhibit symptoms, i.e., preclinical AD (no cognitive impairment) or MCI due to AD (no dementia). An AD-specific example is to aim to delay the onset of cognitive impairment, for example by the removal of amyloid. Finally, tertiary prevention applies to individuals with both pathological signs and symptoms, and should prevent further complications or decline of the disease, i.e., stabilize or improve AD dementia. Depending on how symptomatic is defined, prevention of further decline in MCI due to AD can be considered tertiary prevention (prevention of decline in symptomatic disease), but it can also fall under secondary prevention (delay of the onset of dementia). The scope entails secondary prevention aimed at disease-modification. This means to change the disease course, as opposed to a symptomatic treatment suppressing disease symptoms. The phase 2 proof-of-concept trials is when target engagement needs to be proven.

3.2 Prevention trials using AD biomarker inclusion criteria

Prevention trials with AD biomarker-inclusion criteria emerge from 2009, affecting enrolment and screening procedures (Figure 2). The first prevention trial to require abnormality in a biological marker related to AD in the trial selection criteria is the Lipididiet study, starting March 2009 [48]. Shortly thereafter, in May 2009, another prevention trial in MCI is the first to specifically require evidence of amyloid accumulation, operationalized as either abnormal CSF Aβ, or an abnormal CSF Aβ to tau ratio [49].

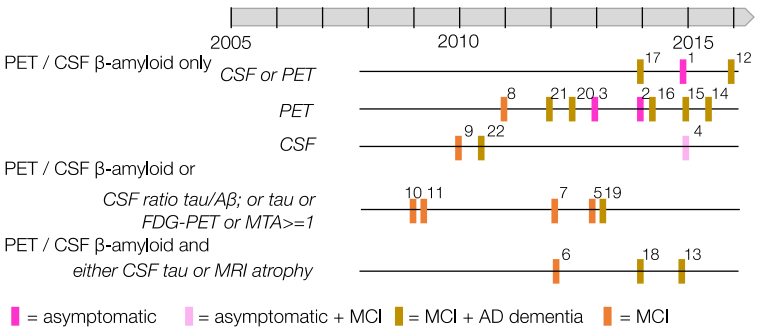

Figure 2 Biomarker inclusion criteria for clinical trials by start date
Every tickmark represents a study: 1) NCT02569398; 2) NCT02008357; 3) NCT02000583; 4) NCT02547818; 5) NCT01953601 6) NCT01522404; 7) NCT01429623; 8) NCT01227564; 9) NCT01224106 10) NCT00890890; 11) NTR1705; 12) NCT02670083 13) NCT02389413; 14) NCT02477800; 15) NCT02322021; 16) NCT02292238; 17) NCT02245737; 18) NCT02054208; 19) ACTRN12613000777796; 20) NCT01767311; 21) NCT01561430; 22) NCT01255163

The requirement of an abnormal amyloid PET scan, aside of an MCI diagnosis, is used for the first time in the trial testing ACC-001+QS21 (active Aβ immunization) in 2011. The first secondary prevention study in cognitively normal individuals with amyloid accumulation is an exercise trial in 2013 [50]. The first pharmaceutical trial in this group is the 'Anti-Amyloid Treatment in Asymptomatic Alzheimer's study' (A4 study) in 2014 [51].

3.3 Challenges of secondary prevention trials in Alzheimer disease

Participants for trials in preclinical AD, such as A4, do not present in large quantities in memory clinics, because most of them do not experience complaints. Therefore, they need to be recruited from the general population, where the biomarker status is unknown. Additionally, trials have strict eligibility criteria on co-morbidities and require a serious commitment from participants. This is a novel challenge for recruitment, finding and screening these individuals, which can lead to major delays in trial completion or even unfinished studies [52] (Ch. 3).

Traditional endpoints include decline on cognitive and functional measures, but in the pre-dementia stages in AD these measures may not be sensitive enough to detect decline over time during the trial [53]. Yet, without a functional endpoint, it is difficult to define the clinical benefit for patients. This is another reason, why a more comprehensive understanding of the total course of AD would be useful to inform clinical trial design and guide the implementation of future treatments. There are two large international consortia, both including academic and private sector partners, aimed at understanding the development of AD dementia and the execution of interventions that play a major role in this thesis, the European prevention of Alzheimer Dementia (EPAD) project and the Dominantly Inherited Alzheimer disease network (DIAN).

4 Consortia in sporadic and autosomal dominant Alzheimer disease

4.1 EPAD project

In 2015, the EPAD project, funded through the Innovative Medicine Initiative (IMI), is initiated with a dual purpose of setting up a framework to execute secondary prevention trials and in parallel study pre-dementia AD [53, 54]. The goal is to set up a platform trial structure, which allows multiple compounds to be investigated according the same protocol. In a platform-trial, sponsors can share placebo-groups, and less participants are needed per study. Additionally, individuals are first included in a 'trial-ready' cohort, in which they are phenotyped, with clinical and cognitive tests, neuroimaging and blood and CSF collection, and are followed over time. About 25% of participants in the trial-ready cohort are expected to participate in a clinical trial during the time frame of the project. Data collected in the trial-ready cohort may be used as run-in data to increase the power of the trial.

In addition, individuals for the trial-ready cohort should be recruited from other studies, enabling preselection. As part of this thesis, we investigate this novel method

of participant recruitment for AD studies. We set up a virtual registry, to which the pre-existing studies can be linked, and have participants that qualify join the EPAD trial-ready cohort (Ch. 3). The idea is that this approach results in less recruitment delay and less screen failures.

4.2 DIAN project

In 2008, the DIAN project starts collecting data for its observational study of carriers of an autosomal dominantly inherited genetic mutation of AD and their family members [55]. In this form of AD, the age of onset of dementia is usually between 40 and 50 years of age [56]. As the age of symptom onset is similar within the mutation type, we can use the estimated years to symptom onset (EYO) as an alternative time scale (irrespective of mutation status). This allows exact staging of individuals including the pre-symptomatic persons, and that is not yet possible in sporadic AD. For example, if someone is 35 years and for the mutation in their family, the average age of onset of dementia is 50, the EYO is minus 15.

The participants undergo regular clinical and cognitive tests, neuroimaging and blood and lumbar puncture for CSF [57]. All family members were included, such that noncarriers are a natural control group. Figure 3 shows how we compare mutation carriers, and non-carrier family members over the disease trajectory. Previous work in this study demonstrated divergence between mutation carriers and noncarriers in CSF Aβ more than 20 years, CSF tau 10 years, and memory decline seven years before dementia onset [57]. The DIAN project also encompasses an intervention study, which is shaped as a platform clinical trial structure and started in 2012 with the first two trial arms [58]. Results of the DIAN observational study are used to design that trial. We use the data of the DIAN observational study to investigate when and how grey matter network change in autosomal dominant AD (ADAD). As disruptions of structural grey matter networks are seen early in sporadic AD, these networks may provide an alternative endpoint for clinical trials in pre-dementia AD. Therefore, we attempt to validate those findings in this pure form of AD, and also investigate the biological correlates of grey matter networks (Ch. 4).

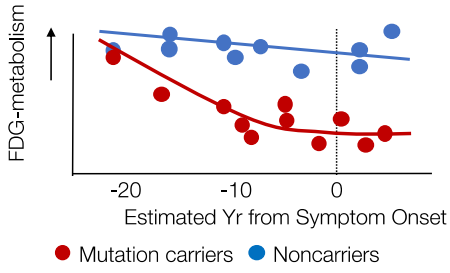

Figure 3 Illustration of comparison by years to symptom onset
Adapted from Bateman et al. NEJM [57]

5 Aim and outline

The purpose of the thesis is to use biomarker and clinical measurements to provide new input into how clinical trials should be structured that aim to evaluate novel secondary prevention strategies for AD. This includes the duration of pre-dementia AD and influencing factors, recruitment and selection of participants, and the development of endpoints to measure treatment response in trials.

The studies address three specific aims:

1. Improve the understanding of the clinical course of Alzheimer disease (2.1,2.2).
2. Set up the EPAD virtual registry for participant recruitment for the EPAD trial-ready cohort and trials, and evaluate learnings (3.1,3.2).
3. Understand how grey matter networks change with disease progression and identify biological correlates in autosomal dominant Alzheimer disease (4.1,4.2).

5.1 Thesis outline

First, we tie together the short-term follow-up of individuals of all AD clinical stages, using the multi-state model technique, to estimate the duration of each stage and of the complete disease course, which can provide information on prognosis (2.1). In the second chapter, we investigate the value of AD biomarkers for the prognosis of a clinically diverting group. Individuals with initially mild cognitive impairment, who improved to normal cognition were continued to be followed on clinical markers. This group is known to be at an increased risk for dementia, and we hypothesize that the underlying cause was AD (2.2). The second topic of this thesis is the set-up of EPAD Registry, a project for linking existing cohorts to enable engagement and selection of participants for EPAD cohort study and secondary prevention trials, and we then evaluate this novel method (3.1,3.2). The final topic addresses changes of structural grey matter networks over the course of AD, to study their use as a potential clinical trial endpoint. We investigate if findings on grey matter network disruptions in sporadic AD translate to individuals with autosomal dominant AD (4.1), and which biological processes, as measured in CSF, may be underlying the disruptions (4.2).

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2

Chapter 2

Clinical course of Alzheimer disease

Chapter 2.1

Duration of preclinical, prodromal, and dementia stages of Alzheimer disease in relation to age, sex, and *APOE* genotype.

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Abstract

INTRODUCTION: We estimated the age-specific duration of the preclinical, prodromal and dementia stages of AD, and the influence of sex, setting, *APOE*, and CSF tau on disease duration.
METHODS: We performed multi-state modeling in a combined sample of 6 cohorts (n=3,268) with death as the end-stage, and estimated the preclinical, prodromal and dementia stage duration.
RESULTS: The overall AD duration varied between 24 years (age 60) and 15 years (age 80). For individuals presenting with preclinical AD, age 70, the estimated preclinical AD duration was 10 years, prodromal AD 4 years, and dementia 6 years. Male sex, clinical setting, *APOE* ε4 genotype and abnormal CSF tau were associated with a shorter duration and these effects depended on disease stage.
DISCUSSION: Estimates of AD disease duration become more accurate if age, sex, setting, *APOE* and CSF tau are taken into account. This will be relevant for clinical practice and trial design.

1 Introduction

Alzheimer disease (AD) is highly prevalent, and a major cause of dementia and death in elderly individuals [1-3]. Accumulation of amyloid in the brain is believed to be the first sign of the disease and can precede a clinical diagnosis of dementia by up to 20 years [1, 4, 5]. Based on the degree of cognitive impairment, AD is often divided into three stages: the preclinical stage, characterized by normal cognitive ability, the prodromal stage, characterized by mild cognitive impairment (MCI), and the dementia stage, with functional impairment [6-9], but it is unclear how long individuals with amyloid pathology spend in each stage. A better understanding of the stage-specific duration of AD is needed to inform patients, caregivers, and clinicians. This information is also useful for the design of clinical studies, as well as to provide context for the interpretation of trial results, in particular the clinical trials that include individuals in pre-dementia stages and aim to slow down progression to AD dementia.

Attempts to quantify the duration of AD should be age-specific, because age imposes the greatest risk for both dementia and mortality, and take into account *APOE* genotype, sex, and cerebrospinal fluid (CSF) tau levels [4, 6, 10-12]. Setting is also important, as progression from MCI to dementia was longer in research settings than in clinical settings [13]. Previous studies on the length of the AD dementia stage reported a duration of 3 to 10 years [14, 15]. Younger age, female sex and lower CSF total tau (tTau) were found to be associated with a longer duration of the AD dementia stage, while the effect of *APOE* genotype was equivocal [14-17]. The median duration of prodromal AD was three years in a pooled memory clinic cohort study, but no age-specific estimates were provided and mortality was not taken into account [18]. The patients with prodromal AD and increased CSF tTau levels tended to

convert sooner to AD dementia [19, 20]. The duration of the preclinical AD stage has been estimated in combination with the prodromal AD stage, which was 17 years, based on extrapolations of change in positron emission tomography (PET) amyloid load over time [21].

We estimated disease duration by applying a multi-state modeling approach, which has been previously used in AD research [22-25], and can offer an estimate of disease duration based on stage progression and mortality rates in the absence of very long follow-up duration. The aim of this study was therefore to estimate the disease duration for preclinical, prodromal and AD dementia stage according to age, setting (clinical versus research), sex, *APOE* genotype, and baseline CSF tTau levels.

2 Methods

2.1 Participants

Six longitudinal cohort studies, including three memory clinic cohorts (Amsterdam Dementia cohort (ADC), DESCRIPA, and ICTUS), and three research cohorts (Alzheimer Disease Neuroimaging Initiative (ADNI), Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing (AIBL) and Prospective Population Study of Women in Gothenburg H70 (Gothenburg H70)), provided data for the study (Supplement A for more cohort information) [26-31]. From these cohorts, we selected participants aged 50 years and older with evidence of amyloid accumulation, and with information on diagnosis and/or mortality at follow-up available. Evidence of amyloid pathology was an inclusion criterion for this study, defined by at least one abnormal marker of amyloid accumulation. The amyloid PET scans were visually rated or a published threshold was applied and for CSF amyloid-beta 1-42 ($A\beta_{42}$) cohort-specific thresholds were applied (Supplement A). In absence of amyloid measures for the ICTUS cohort, only the patients with a clinical diagnosis of AD-type dementia were included and analyses repeated without this cohort. All studies were approved by an ethical review board and their participants gave informed consent.

2.2 AD stages

AD was categorized into four clinical stages: preclinical AD, prodromal AD, mild AD dementia, and moderate to severe AD dementia (from here on shortened to moderate AD dementia). Preclinical AD was defined by amyloid accumulation and normal cognition (Supplement A). Prodromal AD was in this study defined by amyloid accumulation and a diagnosis of MCI, amnestic and non-amnestic [9, 32, 33]. AD dementia was diagnosed according to the NINCDS-ADRDA criteria, and if an amyloid evaluation was available this had to be confirmative [7]. AD dementia was subdivided in mild AD dementia (Clinical Dementia Rating (CDR) below 2, or CDR sum of boxes (CDR-SOB) <10, or (if no CDR was available) MMSE>20), and moderate AD dementia (CDR>1, CDR-SOB>9, or (if no CDR was available) MMSE<21) [34, 35].

2.3 Mortality assessment

The ADC cohort mortality data were obtained from the Dutch population register, while the other studies provided mortality data recorded during the study. In AIBL the exact mortality date of those who died was unknown (n=19) and therefore set at the next planned visit, which is 1.5 years after the last follow-up. In others cases of a missing mortality date (n=4), the date was set 2 years after last follow-up.

2.4 Predictor variables

For all participants, age, sex and setting were available. The setting was classified as clinical for ADC, DESCRIPA and ICTUS and research for ADNI, AIBL and Gothenburg H70. *APOE* genotype was dichotomized according to the presence or absence of the AD-associated ε4 allele of *APOE* and was available in all cohorts except ICTUS. Baseline CSF tTau was classified as normal or abnormal by applying the cohort-specific cut-off and available for the ADC, DESCRIPA, ADNI and Gothenburg H70 studies (Supplement A).

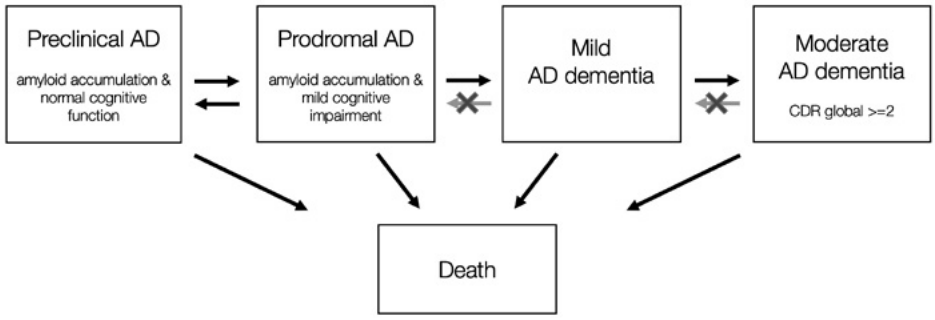

Figure 1 Multi-state Model
Arrows indicate fitted progression and reversion rates between stages in the multi-state model. Moderate to severe AD dementia is shortened to moderate AD dementia for readability.

2.5 Statistical analyses

Baseline characteristics between diagnostic groups were compared using Chi-square, Kruskal-Wallis or ANOVA tests with Tukey post-hoc, where appropriate. To estimate the disease duration, a multi-state model (MSM) with the four stages of AD and death as the end-stage was fitted [36]. All transition rates between stages were incorporated in one model (Figure 1). Reversions from prodromal to preclinical AD were also included in the model. Reversion in the dementia stages were fitted using misclassification (see Supplement B for additional methods and specifications of multi-state model analysis).

Multi-state models with different numbers of covariates were fitted to the data. Age was a time-dependent covariate, and centered at age 70. For each covariate a hazard ratio was calculated for each transition. As most covariate effects on mortality

were not estimable; a restricted model was applied. The first model included only age as covariate, the second model included setting as well, and the third model had age, setting, and sex as covariates. The fourth model included age, setting, and *APOE*, while the fifth model had age, setting, and tau as covariates, and the sixth model included all five covariates. As not all covariates were available for all participants, the number of participants varied between models. The resulting transition rates and hazard ratios are based on every observation of every participant in combination with the time in between the observations.

In a second step, using the MSM maximum likelihood estimate as input, the duration for every stage was estimated. Confidence intervals of 95% were derived by simulation using the asymptotic properties of the maximum likelihood estimation, which allowed comparison between age-specific estimates for the different covariates. R-packages msm for the multi-state transition model and ELECT version 0.3 (Estimating Life-Expectancies for interval censored data) were used to estimate the duration estimates and confidence intervals [36, 37]. Sensitivity analyses included, aside of fitting all covariates in one model, sequentially removing cohorts from the analysis to ensure results were not driven by a single cohort. We also reran all models in the subset with data on all covariates (n=1518).

3 Results

A total of 3,268 participants were included in the analyses across the six cohorts combined. The mean (SD) age at baseline was 73 (8) years with a range of 50 to 96 years. The mean (SD) number of follow-up years was 2.8 (1.9) with a range of 0.3 to 20 years, and a median (IQR) number of 4 (3-5) visits. Progression to at least one consecutive stage was apparent in 981 (32% of 3,034) participants. Table 1 shows how participants in the baseline stages differed in sex, *APOE* ε4 genotype, abnormal CSF tTau, follow-up length and mortality (Suppl. table B.5 for subgroups with data on *APOE* and CSF tTau available).

3.1 Transition rates

In the model that included age, sex and setting, all transition rates to subsequent disease were significantly influenced by age, except mortality in the preclinical AD stage and progression from prodromal AD to mild AD dementia (Suppl. table B.2 for all estimates of the models). Compared to data collected in a research setting, data from clinical settings was associated with a higher progression rate (HR=4.40 [95% CI, 2.80-6.94]) and reversion rate (HR=1.98 [95% CI, 1.15-3.39]) between preclinical and prodromal AD. Additionally, in the clinical setting the progression rates from the prodromal AD to the mild AD dementia stage (HR=1.48 [95% CI, 1.34-1.92]) and from the mild AD to the moderate AD dementia stage (HR=1.41 [95% CI,1.16-1.72]) were higher. Females had a higher progression rate from mild AD to moderate AD dementia, compared to males (HR=1.24 [95% CI, 1.04-1.47]), while their mortality risk in moderate AD dementia was lower (HR=0.60 [95% CI, 0.46-0.80]).

Table 1 Baseline characteristics according to diagnosis

	Preclinical AD (n = 438)	Prodromal AD (n = 729)	Mild AD dementia (n = 1867)	Moderate to severe AD dementia (n = 234)	p-value overall group difference
Age (years)	73 (7)	72 (7)	73 (9)	75 (10)	<0.01[a]
Male (n)	204 (47%)	417 (57%)	781 (42%)	74 (33%)	<0.01
MMSE (0-30, median (IQR)) (n=3252)	29 (28-30)	27 (26-29)	22 (19-24)	16 (13,19)	<0.01[b]
APOE e4 genotype* (n) (n=1984)	210 (49%)	466 (66%)	554 (71%)	35 (51%)	<0.01
Abnormal CSF total tau* (n) (n=1563)	87 (38%)	346 (57%)	535 (80%)	47 (82%)	<0.01
Follow-up years (median (IQR))	3.8 (2-4.5)	3.9 (2.5-4.8)	2.0 (1.5-2.5)	2.0 (1.2-2.3)	<0.01[c]
Progression to next clinical disease stage (n)	87 (20%)	325 (45%)	569 (30%)	NA	NA
Death at follow-up (n)	12 (3%)	76 (10%)	215 (12%)	54 (23%)	NA
Participants by cohort (n ADC/ ADNI/ AIBL/ DESCRIPA/ Gothenburg/ ICTUS)	40/ 180/ 191/ 23/ 4/ 0	140/ 449/ 73/ 49/ 18/ 0	507/ 224/ 69/ 0/ 1/ 1066	64/ 1/ 3/ 0/ 0/ 166	NA

Mean (SD), unless otherwise specified. In Tukey posthoc: [a] Moderate to severe AD dementia older than the MCI and Mild AD dementia group; [b] All groups significantly different from each other; [c] Normal cognition and MCI longer follow-up than dementia groups * Available in subset of cohorts, *APOE* not for ICTUS.

3.2 AD stage duration according to age, sex, and setting

The predicted total disease duration, based on the model with age, for an individual with preclinical AD at age 70 was 20 years (95% CI, 17-21), consisting of a preclinical stage of 10 years (95% CI, 8-11), followed by a prodromal stage of 4 years (95% CI, 3-5), mild AD dementia for 3 years (95% CI, 2-3), and moderate AD dementia for 3 years (95% CI, 2-3, Table 2). Figure 2A shows for those with preclinical AD a lower predicted overall disease duration at older age, which ranged from 24 years (95% CI, 22-25) at age 60 to 15 years (95% CI, 11-17) at age 80. The duration of preclinical AD at age 70 was shorter in a clinical setting (4 years [95% CI, 3-5]) than in a research setting (11 years [95% CI, 9-13]). In the clinical setting, for individuals with prodromal AD, the stage duration of prodromal AD was also shorter, and while the dementia stage duration for these individuals was equal between settings, more time was spent

in the moderate AD stage (Suppl. table B.7a and b). The estimated total duration with starting stage preclinical AD ranged in the clinical setting 19 years (95% CI, 17-20) at age 60 to 11 years (95% CI, 10-12) at age 80 and in the research setting from 26 years (95% CI, 23-28) at age 60 to 15 years (95% CI, 12-17) at age 80. In females the moderate AD dementia stage duration was longer than in males (e.g. 2.1 years (95% CI, 1.1-3.2, p<0.0001 at age 70 in a clinical setting; Figure 2B, Suppl. table B.3).

Table 2 Estimated stage-specific duration of Alzheimer Disease

Starting stage	Duration, time in years (95% CI)	Age 60	Age 70	Age 80
Preclinical AD	Preclinical AD	13 (10.4, 14.9) †	9.9 (8.4, 11.5)	7.6 (5.6, 9.7) †
	Prodromal AD	4.4 (3.7, 4.8)	4.0 (3.3, 4.7)	3.5 (2.3, 4.5) *
	Mild AD dementia	3.5 (3, 3.8) §	2.9 (2.4, 3.3)	2.1 (1.4, 2.5) §
	Moderate AD dementia	3.5 (2.8, 4.1) §	2.6 (2.1, 3.3)	1.7 (1.1, 2.4) §
	Total duration	24.1 (21.8, 25.4)	19.5 (17.3, 20.8)	15.0 (11.0, 16.9)
Prodromal AD	Preclinical AD	3.2 (2.2, 4.3) ‡	1.6 (1.1, 2.1)	0.7 (0.4, 1.2) §
	Prodromal AD	4.6 (4.0, 5.3)	4.4 (3.9, 4.8)	4.0 (3.4, 4.7)
	Mild AD dementia	4.5 (4.0, 4.9) ‡	3.9 (3.5, 4.2)	3.0 (2.5, 3.4) §
	Moderate AD dementia	4.9 (4.2, 5.5) §	3.9 (3.3, 4.5)	2.7 (2.2, 3.5) §
	Total duration	17.2 (15.8, 18.3)	13.6 (12.7, 14.5)	10.3 (9.3, 11.5)
Mild AD dementia	Mild AD dementia	5.0 (4.3, 5.7)†	4.3 (4.0, 4.7)	3.6 (3.2, 3.9) §
	Moderate AD dementia	6.0 (5.1, 6.7) ‡	4.8 (4.2, 5.5)	3.6 (3.0, 4.5) §
	Total duration	10.9 (10.1, 11.8)	9.0 (8.4, 9.7)	7.1 (6.4, 7.9)
Moderate AD dementia	Moderate AD dementia	6.5 (5.4, 7.5) ‡	5.2 (4.0, 6.0)	4.1 (3.5, 5.1) ‡

Estimates based on model including age as covariate (Model 1 in suppl. table B.2). Moderate AD dementia = Moderate to severe AD dementia. Stage estimates significantly different from estimates at age 70: * p<0.05 † p<0.01; ‡ p<0.001; § p<0.0001.

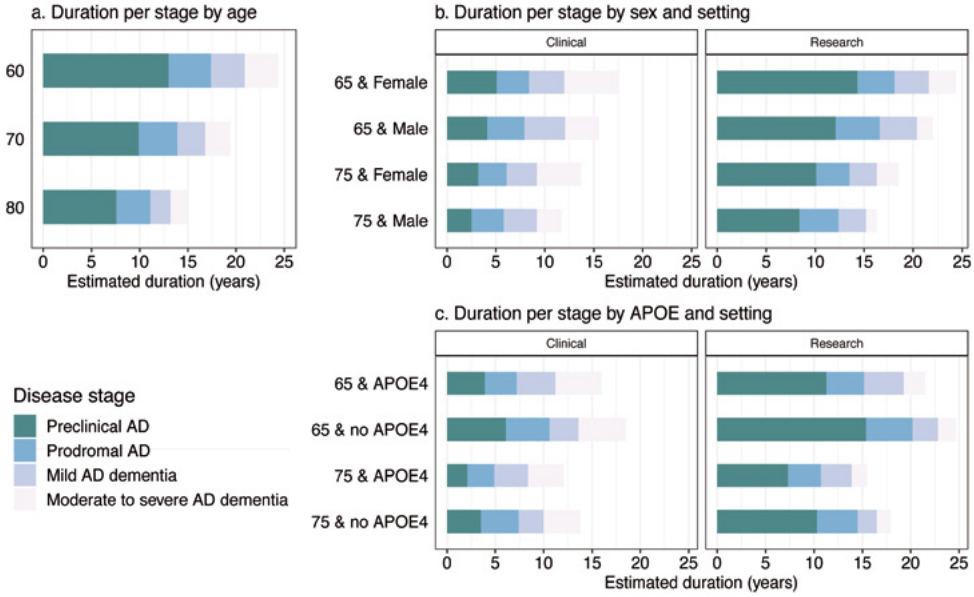

Figure 2 Estimated Stage-specific Duration for Starting Stage Preclinical AD
The panels show the predicted time spend in each stage stacked and stratified for (a) age (model 1); for (b) age, sex, and setting (model 3); and for (c) age, *APOE* genotype, and setting (model 4). Models include age as continues, and (b) sex or (c) *APOE*, and setting as dichotomous covariates. The age refers to the starting stage with preclinical AD and the estimated duration the predicted duration in the subsequent stages in years. The 95% confidence intervals and p-values for estimate comparison can be found for (a) in table 2, for panel (b) in suppl. table B.3, and for panel (c) in suppl. table B.4)

3.3 *APOE* effect

APOE ε4 carriers had, compared to non-carriers, an increased rate of progression from the preclinical AD to prodromal AD stage (HR=1.63 [95% CI, 1.11-2.41]) and from the prodromal AD to mild AD dementia stage (HR=1.50 [95% CI, 1.18-1.90]), and a trend for slower decline from the mild to the moderate AD dementia stage (HR 0.77 [95% CI, 0.60-1.00]). When compared to a non-carrier, an *APOE* ε4 carrier aged 70 in the clinical setting had a 1.6 years (95% CI, 0.4-3.3; p=0.0295) shorter estimated preclinical AD stage duration, and 1.1 years (95% CI, 0.3-2.1; p=0.0110) shorter prodromal AD stage duration, but 1.0 year (95% CI, 0.3-1.8; p=0.0050) longer mild dementia stage duration (Suppl. table B.4). Figure 2C shows how the total predicted disease duration ranged from 12 to 25 years depending on *APOE* ε4 genotype, age and setting.

3.4 Tau effect

As normal CSF tTau level may become abnormal over time only the estimated duration of the starting stages are presented in Table 3. Individuals with preclinical AD and abnormal CSF tTau showed a trend for an increased progression rate from preclinical to prodromal AD (HR=1.49 [95% CI, 0.95-2.35]). In prodromal AD, abnormal tau associated with a decreased reversion rate to preclinical AD stage (HR=0.41 [95% CI, 0.23-0.71]) and increased progression rate to the mild AD dementia stage (HR=1.91 [95% CI, 1.48-2.48]). The estimated preclinical AD stage was shortened by around 3 years and the prodromal AD stage by around 2.5 years (Table 3). There was no association of baseline abnormal tTau with the duration of the dementia stages.

Table 3 Estimated stage-specific duration stratified for baseline CSF total tau by setting at age 70 years

		Clinical setting			Research setting		
Starting stage	Duration, in years (95% CI)	Tau normal	Tau abnormal	Difference (95% CI; p-value)	Tau normal	Tau abnormal	Difference (95% CI; p-value)
Preclinical AD	Preclinical AD	5.6 (3.7, 8.9)	3 (1.9, 4.3)	2.6 (0.7, 5.5; p=0.034)	11.6 (8.3, 14.3)	7.7 (5.6, 9.9)	3.7 (0.4, 7.3; p=0.033)
Prodromal AD	Prodromal AD	5.4 (4.0, 7.0)	3 (2.3, 3.7)	2.4 (1.2, 3.7; p=0.0002)	6.8 (5.5, 8.1)	3.9 (3.3, 4.6)	2.9 (1.4, 4.2; p=0.0001)
Mild AD dementia	Mild AD dementia	4.4 (3.2, 5.9)	3.6 (2.9, 4.4)	0.8 (-0.4, 2.2; p=0.230)	6.4 (4.7, 7.9)	5.4 (4.2, 6.5)	1.1 (-0.5, 2.7; p=0.197)
Moderate AD dementia	Moderate AD dementia	4.9 (3.1, 7.7)	5.9 (4.1, 8.7)	-0.9 (-3.0,1.6; p=0.439)	2.8 (1.8, 4.1)	3.5 (2.5, 4.7)	-0.6 (-2.0, 1.0; p=0.438)

Tau = baseline CSF total tau. Abbreviations: Moderate AD = moderate to severe AD. Estimates based on model including age as continues and baseline CSF tTau and setting as dichotomous covariates (Model 5 in suppl. table B.2).

3.5 Sensitivity analyses

Consecutively removing each of the cohorts did not affect the estimates (Suppl. table B.6). When all variables were combined in one model, most estimates remained unchanged. In the additional analysis of the same models in the subset of individuals with all covariates (n=1518, see Suppl. Table B.8), the effects were similar. Varying the mortality assumptions for unknown mortality dates of those who died, did not change the results.

4 Discussion

We estimated the duration of the preclinical, prodromal, mild dementia, and moderate dementia stages of AD using a multi-state model. Depending on age, sex, *APOE* genotype, baseline CSF tTau and setting, the total disease duration varied between 12 and 25 years, the preclinical stage between 2 and 15, the prodromal stage between 3 to 7, mild AD dementia stage between 2 and 6 and moderate AD dementia stage between 1 and 7 years.

4.1 Effect of age

Age had the strongest effect on the duration of the preclinical and dementia stages, which could be explained by higher progression and mortality rates. The decrease of disease duration of the preclinical AD stage could also be due to a reduction in resilience to AD pathology at higher age, for example due to co-morbid brain disorders, resulting in a faster clinical progression [38]. Alternatively, older individuals may have spent a longer period in the preclinical AD stage before inclusion in the study. Our estimated duration of the combined preclinical and prodromal stage for a 70-year-old (17 years) was very similar to the estimated duration of 17 years pre-dementia AD based on differential equation modeling of the amyloid accumulation rate in individuals aged 72 years on average [21].

4.2 Effect of setting

The shorter duration of the preclinical and prodromal stage in the clinical compared to the research setting could be explained by the fact that individuals who present in a clinical setting are in a more advanced stage of the disease. An alternative explanation is that individuals who present in a clinical setting have a more aggressive disease form of the disease, whereas those with a slower progressive variant would be picked up in the research setting [39]. The estimated differences between settings may be underestimated in the current study, as part of the individuals from the AIBL and ADNI research cohorts were recruited in memory clinics. The effects of setting on disease progression are consistent with other AD studies [40, 41].

4.3 Effect of *APOE* genotype

The shorter age-specific duration of the preclinical stage in *APOE* ε4 carriers is consistent with the observed earlier onset of dementia due to AD in epidemiological studies and the faster cognitive decline of *APOE* ε4 carriers with preclinical AD in research studies [11, 42-44]. While the prodromal stage was shorter in *APOE* ε4 carriers, the dementia stage was longer which would imply that the total symptomatic disease duration is similar, but differently divided over the stages. These findings are important for clinical trials. For example, exclusion of ε4 carriers during a trial, what happened in the high-dose group of the BAN2401 trial, may affect rate of progression and possibly the power of the study [45].

4.4 Effect of sex

The dementia stage duration was longer in women, which was driven by lower mortality in this group. The study did not reveal significant sex differences in the duration of preclinical and prodromal AD stages.

4.5 Effect of tau

The presence of increased CSF tTau was associated with a shorter pre-dementia disease duration, which confirms that increased tau is associated with faster disease progression. Unlike previous studies, no effect of tau on mortality and duration of the AD dementia stage were found, which may be explained by dichotomization of CSF tTau in our analysis [16, 17].

4.6 Duration and mortality

The estimation of total disease duration estimates were in some cases longer than the residual life expectancies of population data [46]. For example, the residual life expectancy at age 80 was reported to be 8-10 years in the USA and Australia (data from 2010-2012), while in our study this ranged from 4 years for those with moderate AD to 15 years for individuals with preclinical AD. One explanation for the longer duration is that we may have overestimated disease duration because mortality had not been checked systematically in all studies. On the other hand, mortality rates in our study cohorts may also be lower because both volunteers participating in studies and memory clinic patients may be healthier at study entry than individuals not participating in research or attending memory clinics.

4.7 Strengths and limitations

A strength of the study is the large sample of participants with amyloid accumulation. The multi-state model approach is another strength, because it enabled the incorporation of multiple clinical stages, including fluctuations between stage, and the mortality risk in a data driven manner. A limitation of the modeling approach is the underlying assumption that progression risk is independent on the previous time spend in a stage, while progression risk may actually change after being in a stage for a longer period of time. This was addressed by taking age as the time-dependent covariate, which has been applied before to overcome this issue [22, 47]. To estimate the disease duration, we had to combine data of multiple cohorts across the disease spectrum. As such, the sample consisted of over 3000 individuals, still not all the effects were estimable. Combining cohort data leads to heterogeneity, i.e. due to different application of diagnostic criteria, cognitive testing and amyloid status. Another limitation was that amyloid status and *APOE* genotype were unknown for AD-type dementia patients of the ICTUS study, but the sensitivity analysis without the ICTUS, yielded very similar results. Additionally, we used the old criteria for the preclinical AD definition, while the recent research criteria also require tau

positivity [8]. Finally, our sample is not representative of the general population, but may be representative of the patients who physicians need to inform, and volunteers that participate in clinical trials.

4.8 Implications

Our estimates are of practical use to clinicians needing to provide prognostic information to research participants and patients. For instance, in a research study with disclosure of abnormal amyloid status, these estimates can give an indication of the prognosis, often asked for by the trial participants before joining the study. The estimates of AD duration are also useful to define target populations for trials. Furthermore, these estimates can be used to indicate how a preventive treatment in the early stage of the disease could impact total disease duration.

4.9. Conclusion

We provided age-specific disease estimates of the duration of AD, including the long pre-dementia stage, according to setting, sex, *APOE* genotype, and presence of tau pathology. Our findings will be useful to provide patients a prognosis, to inform clinical trial design, and can help to model how interventions in early stage AD may influence long-term outcome.

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Alzheimer Disease Neuroimaging Initiative refers to: Data used in preparation of this article were obtained from the Alzheimer Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp,content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf
AIBL Research Group refers to: https://aibl.csiro.au/about/aibl-research-team
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Declarations

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Supplemental data Chapter 2.1

Supplementary file A. Cohort Information

Table A1 Eligibility, diagnostic criteria and amyloid measures for all cohorts

Cohort	ADC	ADNI	AIBL	DESCRIPA	Gothenburg	ICTUS
Age range	>50	55-90	>60	>55	70-84	>50
Participants	Consecutive memory clinic patients	Research volunteers and memory clinics	Research volunteers and memory clinics	Consecutive memory clinic patients	Population based women study	Consecutive GP and memory clinic patients
Relevant exclusion criteria	None	Other disorder causing cognitive impairment; medication causing cognitive impairment, Hachinski >4, GDS>6	Good general health with no history of stroke or other neurological disease	Other disorder causing cognitive impairment	None	MMSE <10, nursing home at entry, pathology leading to <2 years' life expectancy, no caregiver.
Dementia diagnosis	According to criteria NINCDS-ADRDA criteria applied in clinical work-up	Consensus committee applies criteria NINCDS-ADRDA criteria	NINCDS-ADRDA criteria for probable AD and CDR of 1 or more	NINCDS-ADRDA criteria, checked by consensus committee	NINCDS-ADRDA	Probable AD according NINCDS-ADRDA criteria
Criteria for MCI	Petersen's criteria until 2012, thereafter National Institute on Aging-Alzheimer's Association (NIA-AA) criteria for MCI.[4,5]	Memory complaint by subject or study partner, verified by a study partner; below cut-off on Logical Memory II DR Wechsler Memory Scaled (LMII-DR of WMS), education adjusted; MMSE 24-30 (inclusive); CDR = 0.5, Memory score >= 0.5; diagnosis of AD dementia is not met.	Subjective and objective cognitive difficulties in the absence of significant functional loss and had a CDR of < 1 [4,5]	Cognitive test score <1.5 SD, dementia criteria not met.	Winblad criteria[5]	NA

Criteria for Cognitively normal	Criteria for MCI and dementia not met and no current psychiatric illness.	Normal scoring on Logical Memory II subscale (delayed Paragraph Recall) Wechsler Memory Scaled (LMII-DR of WMS), education adjusted, MMSE 24-30, CDR = 0; no significant impairment in cognitive functions or ADL.	Criteria for MCI and dementia not met, enrichment with: wide age range, 50% memory complaints, 50% *APOE* ε4	No cognitive test score <1.5 SD, dementia criteria not met.	Criteria for MCI and dementia not met.	NA
Amyloid pathology measures	Visually rated positive on amyloid PET (PiB or Florbetaben) by experienced raters, or CSF $A\beta_{42}$ below 640 ng/L on the Innotest assay.[2]	Positive on amyloid PET scans by cut-offs were for PiB 1.5 SUVR for Florbetapir 1.11[1] SUVR or CSF $A\beta_{42}$ below $A\beta_{42}$ 192 ng/L of the Luminex assay[1,3]	Positive on amyloid PET PIB SUVR > 1.5	CSF $A\beta_{42}$ below 550 ng/L on the Innotest assay.	CSF $A\beta_{42}$ below 640 ng/L cut-off on Innotest assay.	No amyloid measures available
Tau pathology measures	Above CSF tTau> 375 pg/ml on the Innotest assay.	Above CSF tTau> 92 pg/ml on the Luminex assay	NA	Above CSF tTau> 375 pg/ml on the Innotest assay.	Above CSF tTtau> 375 pg/ml on the Innotest assay.	NA

Table A2 Participants numbers and baseline characteristics of participants by cohort

	ADC (N=751)	ADNI (N=854)	AIBL (N=336)	DESCRIPA (N=72)	Gothenburg (N=23)	ICTUS (N=1232)
Baseline Diagnosis						
Normal cognition, No.	40	180	191	23	4	0
Mild Cognitive Impairment, No.	140	449	73	49	18	0
Mild AD dementia, No.	507	224	69	0	1	1066
Moderate to severe AD dementia, No.	64	1	3	0	0	166
Follow-up, y median (IQR)	3 (1.5-4.5)	3 (2-4.2)	4.5 (1.5-4.5)	2.5 (2-3)	12 (8-16)	2 (1.5-2)
Age, y mean (SD)	66 (7)	74 (7)	74 (7)	69 (8)	74 (4)	77 (7)
Female, %	50	45	51	46	100	65

ADC=Amsterdam Dementia Cohort; ADNI = Alzheimer's Disease Neuroimaging Initiative; AIBL = Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing; Gothenburg = Prospective Population Study of Women in Gothenburg.

Table A3 Total amyloid positive participants and numbers excluded by cohort

	ADC	ADNI	AIBL	DESCRIPA	Gothenburg	ICTUS
Amyloid positive	751	882	418	101	23	n/a
After removal duplicate cases of ADC or no Dx	n/a	n/a	418	83	n/a	1301
N included with FU	751	854	336	72	23	1232

For ADNI number of individuals is at download date. ADC is a clinical database, which was recently updated, so numbers cannot be traced back

Table A4 Overview characteristics included versus not included due to no follow-up by cohort and baseline diagnosis

		CN		MCI		Dementia	
		No follow-up	With follow-up	No follow-up	With follow-up	No follow-up	With follow-up
ADNI	N	2	180	11	449	15	225
	Age	75.3 (1.1)	74.8 (6.0)	72.0 (9.1)	73.3 (7.2)	77.8 (9.9)	74.2 (7.9)
	Female, N (%)	2 (100%)	101 (56%)	6 (55%)	185 (41%)	6 (40%)	97 (43%)
	MMSE	29.5 (0.7)	29.0 (1.2)	27.3 (2.3)	27.5 (1.8)	22.9 (2.1)	23.3 (2.0)
AIBL	N	24	191	35	73	23	72
	Age, mean (SD)	71 (4.5)	73.4 (7.0)	73.3 (5.8)	76 (6.4)	75.7 (7.7)	73.4 (8.1)
	Female, N (%)	13	94	13	35	10	41
	MMSE, mean (SD)	28.6 (1.1)	28.6 (1.3)	26.3 (2.5)	26.6 (2.3)	20.7 (5.2)	20.8 (4.9)
DESCRIPA	N	2	23	9	49	n/a	n/a
	Age, mean (SD)	71 (6)	69 (9)	71 (8)	70 (8)	n/a	n/a
	Female, N (%)	0 (0%)	14 (61%)	5 (56%)	19 (39%)	n/a	n/a
	MMSE, mean (SD)	28.5 (0.7)	28.7 (1.3)	26.0 (2.5)	26.5 (2.8)	n/a	n/a
ICTUS	N	n/a	n/a	n/a	n/a	69	1232
	Age	n/a	n/a	n/a	n/a	75.5 (7.7)	76.7 (7.4)
	Female, N (%)	n/a	n/a	n/a	n/a	52 (75%)	802 (65%)
	MMSE	n/a	n/a	n/a	n/a	19.8 (4.2)	20.4 (4.0)

Not relevant for ADC and Gothenburg, because all had follow-up data.

ADNI methods

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD).

References supplement A

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Supplement B.
Methods and Specifications Multistate Model Analysis and Estimations of Disease Duration

Background multistate model and disease duration

A multistate model is a Markov model in which multiple transition rates can be estimated in a single model, while also allowing non-linear rates over time with age as a time-dependent covariate (i.e. being age-specific). This technique was previously used in AD research to estimate age-related AD biomarker abnormality prevalence and to extrapolate the effect on the prevalence if a preventive treatment would come available (Jack et al. 2016, Brookmeyer et al. 2018). The multistate model was fit with the R-package msm (Jackson, 2011). After determining the transition rates, the maximum likelihood estimate can be used as input for predicting the duration for every stage, as well as to derive 95% confidence intervals by simulation using the asymptotic properties of the maximum likelihood estimation. These calculations were done with the R-package created by Ardo van den Hout called Estimating Life-Expectancies for interval censored data (ELECT) (van den Hout 2017, Jackson 2011). P-values of differences of the duration estimates between covariates specified in a model were obtained with the same software. More specifically each of the simulations were subtracted between two groups of a fitted model (i.e. male vs female) to derive a 95% confidence interval of the difference, and then calculate the p-value of the estimate. The same seed was set for all simulations to assure the same samples were drawn from the same multivariate distribution. We build up several models with the goal to estimate disease durations and investigate the effects of certain covariates. This supplement describes the data input and the choices in more detail.

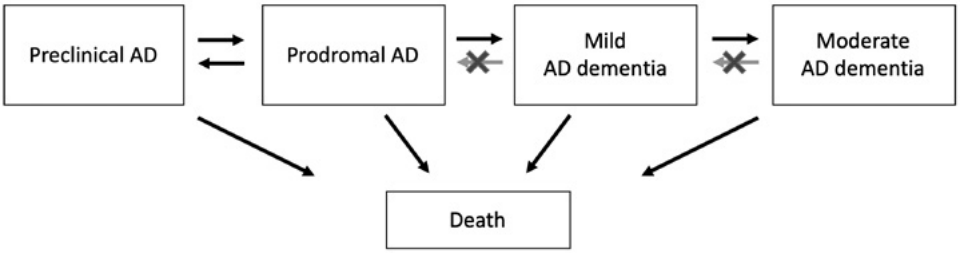

Figure B.1 Five stage multistate model

Rationale of model choice

Data on clinical diagnosis and survival at every follow-up visit were used to fit a multistate model that included five stages. This model contained four living stages: preclinical AD, prodromal AD, mild dementia, and moderate to severe dementia. Death was the end-stage (Figure B1). Reversion from prodromal AD to preclinical AD was kept in the model as MCI is a clinically defined syndrome based on test scores, from which a participant can at least temporary improve, even in the presence of amyloid pathology (n=62 in this dataset). As a result, we report a duration in the preclinical stage for participants with prodromal AD at baseline. Reversions from mild dementia to prodromal AD or from moderate to mild dementia were treated as being misclassified in the more severe stage previous to the reversion, because it was considered that these reversions were due to variability in clinical scores rather than improvement of the disease. The probabilities for misclassifications were low; with 0.014 (95% CI, 0.010-0.021) of true state prodromal AD being misclassified as mild AD

dementia and 0.043 (95% CI, 0.037-0.049) of true state mild AD being misclassified as moderate to severe AD dementia. Few participants with preclinical or prodromal AD received during follow-up a clinical diagnosis of non-AD dementia at follow-up (n=10), and were classified as having mild or moderate to severe dementia based on the global CDR score.

Specifics of data

Table B.1a shows the state table of the dataset. This table contains all observations. Each individual can have multiple observations. 'From' does not refer to baseline diagnosis, but to diagnosis at previous visit. The time interval between visits varies. Table B.1b-d present the number observations at each moment in time, the number of observations per individual and the number of observations per stage.

Basic model specifications

The baseline estimates (transition rates) were centered at age 70. First the hazard ratios per year increase in age were estimated in Model 1 of which the estimates are in table B.2 below. Here the transition rates are defined for age in years. Based on these models, we estimated the duration of stages in Table 2 according to age. In the multistate model the rate for transitioning out of a state can be based on more than one rate. For instance the rate for moving from preclinical AD is based on the rates of preclinical AD to prodromal AD and of preclinical AD to death. In this case, the rate for preclinical AD to prodromal AD should not be interpreted in isolation. Interpretation of a fitted model is typically done using hazard ratios, as presented in the manuscript.

Table B.1a Summary of all transitions – Multistate model state table

To / From	CN	MCI	Mild AD dementia	Moderate AD dementia	Death	End of follow-up
CN	1094	105	9	0	13	70
MCI	72	1819	344	11	31	133
Mild AD dementia	0	17	3787	684	187	620
Moderate AD dementia	0	0	124	782	135	192

Table B.1b Number of observations per follow-up time
Table B.1c Number of observations per individual
Table B.1d Number of observations per stage

B.1b

Follow-up, y	0	<0.3	0.5	1	2	3	4	5	6	7	8	9	10-20
Observations, No.	3268	20	2071	2381	3228	1034	535	508	190	100	80	35	38

B.1c

Observations, No.	2	3	4	5	6	7	8	9	10	11	12	13
Participants, No.	658	497	813	870	213	107	41	20	17	17	8	7

B.1d

Stage	CN	MCI	Mild AD dementia	Moderate AD dementia	Death	Last known alive, diagnosis unknown
Observations, No.	1604	2670	6131	1711	357	1015

Models with covariates sex and setting

We build up the model by adding the effects setting and then of sex, shown in Table B.2, model 2 and 3. As there is a covariate effect on every transition, the number of parameters increases rapidly when adding covariates to a model. In particular the point estimates of effect of covariates on the transitions from preclinical AD, prodromal AD and mild AD dementia to death were not estimable, leading to incredibly large or small hazard ratios with confidence intervals of more than 3 times the hazard ratios. The only exception was the transition from mild AD dementia to death for sex in model 3. The others were omitted.

Model with APOE

We next performed the analysis with *APOE* ε4 as predictor (Table B.2, model 4). In the subset of individuals with *APOE* data (n=1984) the effects of age, sex and setting on stage transitions were not different from those in the full dataset. Sex did no longer predict transition from mild dementia to death. The sample demographics are shown in table B.5a and the prediction of the age only model in table B.6a. The effects of the covariates on death in the preclinical, prodromal and mild AD dementia stage were again omitted because they were not estimable. Model 4 with age, *APOE* and setting was used to generate the estimates with starting stage preclinical AD in Figure 2 and Table B.4.

Model with CSF total tau

We next performed the analysis with baseline CSF total tau as predictor (Table B.2, model 5). In the subset of individuals with baseline CSF total tau (n=1563) data (table B.5a), the effect of age and sex, setting on stage transitions were similar to those in the full dataset. The confidence intervals were wider, and the effect of age and sex on mild AD dementia to moderate AD dementia lost significance. The sample demographics are shown in table B.5b and the prediction of the age only model in table B.6b. Model 5 with age, setting and tau was used to generate the estimates in Table 3. Model 6 includes all covariates and was part of the sensitivity analysis showing similar estimates (Table B.2).

Table B.3a Estimated stage-specific duration for starting stage preclinical AD stratified by sex and setting

Duration in years (CI, 95%)	Age 65 Clinical setting		Age 65 Research setting		Age 75 Clinical setting		Age 75 Research setting	
	Female	Male	Female	Male	Female	Male	Female	Male
Preclinical AD	5.1 (3.6-7.3)	4.1 (3-5.8)	14.3 (11.8-16.8)	12.1 (9.8-14.3)	3.2 (2-5.1)	2.5 (1.6-3.8)	10.1 (7.9-12.4)	8.4 (6.6-10.4)
Prodromal AD	3.3 (2.6-4.1)	3.8 (3-4.6)	3.8 (3-4.5)	4.5 (3.8-5.2)	2.9 (2.2-3.8)	3.3 (2.6-4.3)	3.4 (2.5-4.2)	4.0 (3-4.8)
Mild AD dementia	3.6 (3.1-3.9)	4.2 (3.6-4.7)	3.5 (2.7-4.1)	3.8 (3-4.5)	3.1 (2.6-3.5)	3.4 (2.8-3.9)	2.8 (1.9-3.6)	2.8 (2.1-3.7)
Moderate AD dementia	5.6 (4.5-6.8)	3.4* (2.7-4)	2.8 (2-3.7)	1.6* (1.1-2.1)	4.5 (3.3-5.7)	2.5* (1.8-3.3)	2.2 (1.3-3)	1.1* (0.7-1.5)
Total duration	18 (16-20)	16 (14-17)	24 (22-26)	22 (20-24)	14 (12-15)	12 (11-13)	18 (15-20)	16 (14-18)

Table B.2 All six models with baseline transition rates and hazard ratios (HR)

	Preclinical AD to prodromal AD	Preclinical AD to death	Prodromal AD to preclinical AD	Prodromal AD to mild dementia	Prodromal AD to death	Mild AD to moderate AD dementia	Mild AD dementia to death	Moderate AD dementia to death
Model 1 AGE								
Transition rate, at age 70	0.083 (0.066,0.103)	0.002 (0.001,0.010)	0.049 (0.039, 0.062)	0.199 (0.176,0.223)	0.004 (0.001,0.011)	0.200 (0.181,0.220)	0.004 (0.001,0.014)	0.164 (0.140,0.191)
HR Age, per year increase	1.027 (1.001,1.053)	1.057 (0.897,1.245)	0.951 (0.923,0.979)	1.004 (0.990,1.018)	1.126 (1.027,1.240)	1.011 (1.0001,1.022)	1.163 (1.068,1.268)	1.024 (1.010,1.038)
Model 2 AGE/SETTING								
Transition rate, at age 70	0.060 (0.046, 0.078)	0.003 (0.001,0.010)	0.0407 (0.0300,0.05)	0.1783 (0.1538,0.2068)	0.003 (0.001,0.011)	0.151 (0.125, 0.182)	0.005 (0.002, 0.015)	0.206 (0.1548, 0.274)
HR Age, per year increase	1.047 (1.020,1.073)	1.049 (0.894,1.230)	0.963 (0.933,0.994)	1.011 (0.996,1.027)	1.128 (1.024,1.243)	1.012 (1.001,1.022)	1.148 (1.065,1.238)	1.022 (1.008,1.036)
HR Clinic setting (ref=research setting)	4.832 (3.106,7.519)	-	2.132 (1.259,3.61)	1.450 (1.114,1.887)	-	1.446 (1.188,1.760)	-	0.750 (0.554,1.014)
Model 3 AGE/SEX/SETTING								
Transition rate, at age 70	0.0682 (0.049,0.094)	0.003 (0.001,0.010)	0.040 (0.027,0.058)	0.166 (0.138,0.199)	0.003 (0.001,0.011)	0.137 (0.111,0.168)	0.005 (0.001,0.022)	0.263 (0.193,0.357)
HR Age, per year increase	1.046 (1.020,1.074)	1.054 (0.903,1.231)	0.965 (0.934,0.997)	1.013 (0.998,1.029)	1.127 (1.020,1.245)	1.011 (1.001,1.023)	1.164 (1.067,1.227)	1.025 (1.010,1.040)
HR Female (ref=male)	0.769 (0.534,1.107)	-	1.028 (0.639,1.651)	1.154 (0.930,1.431)	-	1.237 (1.039,1.473)	0.446 (0.169,1.174)	0.602 (0.456,0.795)
Model 4 AGE/*APOE*/SETTING								
Transition rate, at age 70	0.043 (0.032,0.062)	0.002 (0.001,0.009)	0.0427 (0.030,0.066)	0.133 (0.106,0.167)	0.004 (0.001,0.011)	0.196 (0.151,0.255)	0.001 (0.000,0.020)	0.193 (0.127,0.293)
HR Age, per year increase	1.061 (1.033,1.090)	1.0638 (0.906,1.249)	0.963 (0.932,0.996)	1.017 (1.001,1.033)	1.124 (1.025,1.232)	1.004 (0.987,1.021)	1.292 (1.086,1.538)	1.022 (1.0003,1.04)

Clinic setting (ref=research setting)	4.501 (2.786,7.273)	-	1.890 (1.088,3.284)	1.444 (1.101,1.894)	-	1.481 (1.133,1.935)	-	0.704 (0.468,1.060)
Model 5 AGE/TAU/SETTING								
Transition rate, at age 70	0.068 (0.046,0.099)	0.001 (0.000,0.022)	0.0487 (0.033,0.071)	0.115 (0.09,0.145)	0.004 (0.001,0.012)	0.137 (0.099,0.189)	0.001 (0.0004,0.02)	0.284 (0.018,0.450)
HR Age, per year increase	1.035 (1.001,1.071)	1.073 (0.810,1.422)	0.973 (0.940,1.007)	1.011 (0.994,1.028)	1.112 (1.014,1.219)	1.003 (0.984,1.021)	1.274 (1.072,1.513)	1.016 (0.993,1.040)
HR Abnormal baseline CSF tau (ref=normal baseline CSF tau)	1.494 (0.949,2.352)	-	0.407 (0.234,0.709)	1.914 (1.481,2.475)	-	1.225 (0.901,1.664)	-	0.843 (0.557,1.276)
Clinic setting (ref=research setting)	3.166 (1.876,5.342)	-	2.811 (1.563,5.057)	1.332 (1.006,1.764)	-	1.513 (1.125,2.035)	-	0.606 (0.388,0.946)
Model 6 AGE/SEX/*APOE*/ TAU/SETTING								
Transition rate, at age 70	0.079 (0.047,0.134)	0.001 (0.0001,0.02)	0.044 (0.025,0.077)	0.096 (0.071,0.130)	0.004 (0.001,0.012)	0.159 (0.109,0.231)	0.0006 (0.000,0.032)	0.302 (0.176, 0.531)
HR Age, per year increase	1.042 (1.005,1.080)	1.079 (0.825,1.410)	0.976 (0.941,1.013)	1.016 (0.998,1.035)	1.120 (1.017,1.234)	1.005 (0.986,1.024)	1.294 (1.053,1.589)	1.015 (0.991,1.039)
HR Female (ref=male)	0.562 (0.359,0.878)	-	1.072 (0.625,1.838)	0.997 (0.778,1.279)	-	1.120 (0.853,1.444)	-	0.700 (0.487,1.007)
HR *APOE* ε4 carrier (ref=non-carrier)	1.201 (0.756,1.909)	-	1.197 (0.681,2.105)	1.318 (1.010,1.720)	-	0.749 (0.568,0.988)	-	1.117 (0.766,1.628)
HR Abnormal baseline CSF tau (ref=normal baseline CSF tau)	1.470 (0.923,2.340)	-	0.358 (0.199,0.643)	1.846 (1.417,2.405)	-	1.189 (0.866,1.632)	-	0.928 (0.603,1.427)
HR Clinic setting (ref=research setting)	3.335 (1.884,5.905)	-	2.801 (1.522,5.157)	1.368 (1.022,1.830)	-	1.559 (1.1501,2.11)	-	0.587 (0.370,0.931)
HR Clinic setting (ref=research setting)	4.403 (2.793,6.943)	-	1.975 (1.150,3.389)	1.477 (1.134,1.924)	-	1.410 (1.158,1.718)	-	0.771 (0.570,1.045)

Hazard ratios that are different from 1 in bold. Moderate = moderate to severe. ' - ' = the HR was not estimable.

Table B.3b P-values and estimated difference in duration

Difference in years (CI, 95%)	Age 65 Clinical setting Female vs male	p-value	Age 65 Research setting Female vs male	p-value	Age 75 Clinical setting Female vs male	p-value	Age 75 Research setting Female vs male	p-value
Preclinical AD	0.97 (-0.41-2.47)	0.1880	2.13 (-0.83- 5.25)	0.1700	0.67 (-0.27- 1.78)	0.2028	1.68 (-0.53- 4.25)	0.1692
Prodromal AD	-0.53 (-1.28- 0.19)	0.1577	-0.74 (-1.65- 0.03)	0.0842	-0.45 (-1.07- 0.16)	0.1530	-0.63 (-1.38- 0.02)	0.0802
Mild AD dementia	-0.6 (-1.21- 0.04)	0.0590	-0.33 (-1.23- 0.47)	0.4501	-0.28 (-0.86- 0.24)	0.3274	0 (-0.82- 0.69)	0.9911
Moderate AD dementia	2.27 (1.14- 3.37)	0.0001	1.24 (0.54- 1.91)	0.0004	1.99 (1.1- 2.93)	<0.0001	1.06 (0.44- 1.65)	0.0006

Abbreviations: Moderate AD dementia = moderate to severe AD dementia. Model includes age as continues and sex and setting as dichotomous covariates. Based on model 4 in table B.2. Significant difference between estimates between sex, same age and setting: *p<0.0. P-values based on confidence intervals of differences for each stratification based on 500 simulation with the same seed.

Table B.4a Estimated stage-specific duration for starting stage preclinical AD stratified by *APOE* and setting

Duration, time in years (CI, 95%)	Age 65		Age 65		Age 75		Age 75	
	Clinical setting		Research setting		Clinical		Research setting	
	No *APOE* ε4	*APOE* ε4	No *APOE* ε4	*APOE* ε4	No *APOE* ε4	*APOE* ε4	No *APOE* ε4	*APOE* ε4
Preclinical AD	6.1 (4.0-8.6)	3.9* (2.7-5.5)	15.4 (13-18)	11.3* (9.3-13)	3.5 (2.2-5.4)	2.1* (1.3-3.2)	10.3 (8.3-13)	7.3* (5.9-8.8)
Prodromal AD	4.5 (3.4-5.8)	3.3* (2.7-4)	4.8 (3.7-5.6)	3.9 (3.3-4.5)	3.9 (2.9-5.1)	2.8* (2.2-3.6)	4.2 (3-5.3)	3.4 (2.7-4.1)
Mild AD dementia	3.0 (2.2-3.8)	4.0* (3.2-4.8)	2.6 (1.9-3.4)	4.1* (3.2-4.9)	2.6 (1.8-3.4)	3.5* (2.6-4.3)	2.0 (1.3-2.9)	3.2* (2.4-4)
Moderate AD dementia	4.9 (3.3-7.3)	4.8 (3.4-6.5)	1.9 (1.1-2.9)	2.2 (1.5-2.9)	3.8 (2.2-5.9)	3.7 (2.2-5.9)	1.4 (0.8-2.2)	1.6 (1-2.3)
Total duration	18 (16-21)	16 (14-18)	25 (22-26)	22 (20-23)	14 (12-16)	12 (10-14)	18 (15-20)	16 (14-18)

Table B.4b P-values and estimated difference in duration

Difference in years (CI, 95%)	Age 65 Clinical setting No *APOE* ε4 vs *APOE*	p-value	Age 65 Research setting No *APOE* ε4 vs *APOE*	p-value	Age 75 Clinical setting No *APOE* ε4 vs *APOE*	p-value	Age 75 Research setting No *APOE* ε4 vs *APOE*	p-value
Preclinical AD	2.08 (0.53- 4.06)	0.0210	3.97 (0.98- 6.72)	0.0067	1.26 (0.29- 2.67)	0.0383	2.96 (0.69- 5.08)	0.0083
Prodromal AD	1.24 (0.33- 2.26)	0.0117	0.93 (-0.11- 1.90)	0.0698	1.09 (0.31- 1.96)	0.0100	0.81 (-0.13-1.73)	0.0889
Mild AD dementia	-1.06 (-1.86- -0.32)	0.0070	-1.56 (-2.33- -0.79)	0.0001	-0.94 (-1.56- -0.34)	0.0025	-1.21 (-1.77- -0.64)	<0.0001
Moderate AD dementia	0.15 (-1.35- 2.03)	0.8608	-0.27 (-0.99- 0.66)	0.5195	0.14 (-1.11- 1.62)	0.8437	-0.19 (-0.78-0.49)	0.5669

Abbreviations: Moderate AD dementia = moderate to severe AD dementia. Model includes age as continues and *APOE* ε4 and setting as dichotomous covariates. Based on model 5 in table B.2. Significant difference between estimates between genotype, same age and setting: *p<0.05. P-values based on confidence intervals of differences for each stratification based on 500 simulation with the same seed.

Table B.5a Baseline characteristics of participants with *APOE* data classified by baseline AD stage

	Preclinical AD (N = 431)	Prodromal AD (N = 709)	Mild AD dementia (N= 776)	Moderate AD dementia (N = 68)	P-value
Age, year mean (SD)	73 (7)	72 (8)	69 (9)	66 (8)	<0.01
Male, No. (%)	200 (46%)	407 (57%)	394 (51%)	25 (37%)	<0.01
MMSE (0-30), median (IQR)	29 (2)	28 (3)	22 (5)	13 (8.2)	<0.01
APOE ε4 genotype, No. (%)	210 (49%)	466 (66%)	554 (71%)	35 (51%)	<0.01
Abnormal CSF tau^, No. (%)	85 (37%)	328 (56%)	517 (80%)	47 (82%)	<0.01
Follow-up, years median (IQR)	4 (2.5)	3.9 (2.3)	2.5 (3)	3.5 (3)	<0.01
Visits, No. median (IQR)	4 (2)	5 (2)	3 (2)	2 (1)	<0.01
Progression to next stage, No. (%)	86 (20%)	320 (45%)	200 (26%)	NA	NA
Death at follow-up, No. (%)	11 (2%)	68 (10%)	106 (14%)	23 (34%)	NA

Table B.5b Baseline characteristics of participants with baseline CSF tau classified by baseline AD stage

	Preclinical AD (N = 231)	Prodromal AD (N = 607)	Mild AD dementia (N= 668)	Moderate AD dementia (N = 57)	P-value
Age, years mean (SD)	73 (7)	72 (7)	68 (8)	66 (8)	<0.01
Male, No. (%)	98 (42%)	352 (58%)	343 (51%)	22 (39%)	<0.01
MMSE (0-30), median (IQR)	29 (2)	28 (3)	22 (4)	14 (7)	<0.01
APOE ε4 genotype, No. (%)	117 (52%)	383 (65%)	464 (72%)	30 (53%)	<0.05
Abnormal CSF tau, No. (%)	87 (38%)	346 (57%)	535 (80%)	47 (82%)	<0.01
Follow-up, years median (IQR)	3 (2)	3.8 (2.4)	2.5 (3)	3.5 (2.5)	<0.01
Visits, No. median (IQR)	4 (2)	5 (3)	3 (2)	2 (1)	<0.01
Progression to next stage, No. (%)	57 (24%)	270 (44%)	166 (25%)	NA	NA
Death at follow-up, No. (%)	10 (4%)	63 (10%)	98 (15%)	21 (37%)	NA

^ Available in subset of cohorts. Moderate AD dementia = moderate to severe AD dementia.

Table B.6a Predicted stage-specific disease duration – subset with *APOE* or baseline CSF total tau

		Subset with *APOE* (n=1984)*			Subset with CSF total tau (n=1563)^		
Starting stage	Duration, time in years (CI, 95%)	Age 60	Age 70	Age 80	Age 60	Age 70	Age 80
Preclinical AD	Preclinical AD	13.2 (11-15)	10 (8.6-11.5)	7.5 (5.5-9.6)	9.8 (6.9-12)	8.1 (6.6-9.7)	6.6 (4.4-9)
	Prodromal AD	4.4 (3.8-4.8)	4.1 (3.3-4.7)	3.6 (2.4-4.6)	4.8 (3.9-5.4)	4.4 (3.5-5)	3.7 (2.3-4.6)
	Mild AD dementia	3.8 (3.1-4.4)	3.2 (2.6-3.8)	2 (1.3-2.9)	4.2 (3.2-4.8)	3.5 (2.7-4.2)	2.1 (1.3-3)
	Moderate AD dementia	3 (2.3-3.8)	2 (1.4-2.6)	1 (0.6-1.7)	3.3 (2.4-4.1)	2.1 (1.5-2.9)	1 (0.5-1.7)
Prodromal AD	Preclinical AD	3.2 (2.2-4.3)	1.5 (1.2-2)	0.7 (0.4-1.2)	2.5 (1.6-3.4)	1.3 (0.9-1.8)	0.6 (0.3-1)
	Prodromal AD	4.7 (4-5.4)	4.5 (4-4.9)	4.1 (3.4-4.8)	5 (4.1-5.7)	4.7 (4.1-5.1)	4.2 (3.4-5)
	Mild AD dementia	4.5 (3.9-5)	4.4 (3.8-4.9)	3.4 (2.6-4.1)	4.5 (3.9-5.1)	4.4 (3.8-5.1)	3.4 (2.6-4.1)
	Moderate AD dementia	4.6 (3.9-5.3)	3.3 (2.6-4)	1.9 (1.3-2.7)	4.4 (3.6-5.2)	3.1 (2.4-4)	1.8 (1.2-2.7)
Mild AD dementia	Mild AD dementia	4.5 (3.8-5.3)	4.8 (4.2-5.3)	4.3 (3.6-5.1)	4.4 (3.7-5.1)	4.8 (4-5.5)	4.4 (3.5-5.2)
	Moderate AD dementia	6 (5.1-6.9)	4.4 (3.6-5.2)	2.8 (2.1-4)	5.7 (4.8-6.6)	4.2 (3.4-5)	2.7 (1.9-3.7)
Moderate AD dementia	Moderate AD dementia	6.5 (5.4-7.7)	4.9 (4.1-5.8)	3.7 (2.9-5)	6.2 (5.1-7.3)	4.7 (4-5.6)	3.5 (2.7-4.7)

Models with age as covariate. Moderate AD dementia = moderate to severe AD dementia. *All estimates have overlapping confidence intervals with confidence intervals based on the full dataset Table 2. In these subsets no ICTUS data, i.e. only confirmed amyloid positive individuals. ^In this subset no ICTUS and AIBL data.

Table B.6b Predicted stage-specific disease duration – subsequently removing cohorts

		Subset without ADNI (n=2414)			Subset without ADC (n=2517)			Subset without DESCRIPA (n=3196)			Subset without Gothenburg (n=3245)		
Starting stage	Duration, time in years (CI, 95%)	Age 60	Age 70	Age 80	Age 60	Age 70	Age 80	Age 60	Age 70	Age 80	Age 60	Age 70	Age 80
Preclinical AD	Preclinical AD	12.9 (10-15.2)	11 (8.5-14)	8.6 (5.5-13)	15 (12-17)	10.4 (9-12)	7.1 (5.3-8.9)	13.6 (11-15.5)	10.2 (8.7-12)	7.6 (5.6-9.7)	13 (11-14.8)	9.9 (8.4-11)	7.6 (5.7-9.5)
	Prodromal AD	3.2 (2.5-3.7)	2.5 (1.7-3)	1.6 (0.8-2.5)	4.4 (3.6-4.9)	4.1 (3.2-4.7)	3.6 (2.4-4.5)	4.4 (3.6-4.9)	4.1 (3.3-4.7)	3.6 (2.5-4.6)	4.4 (3.7-4.8)	4 (3.3-4.7)	3.5 (2.4-4.5)
	Mild AD dementia	3.5 (2.8-3.8)	2.7 (1.8-3.2)	1.7 (0.8-2.4)	3.4 (2.8-3.8)	2.7 (2.2-3.1)	2 (1.3-2.4)	3.4 (2.9-3.7)	2.8 (2.3-3.2)	2 (1.3-2.5)	3.5 (3.1-3.8)	2.9 (2.4-3.3)	2 (1.3-2.6)
	Moderate AD dementia	4 (3.1-4.7)	2.9 (1.9-3.7)	1.7 (0.8-2.6)	3.1 (2.5-3.7)	2.6 (1.9-3.1)	1.9 (1.2-2.7)	3.4 (2.8-3.9)	2.6 (2-3.2)	1.7 (1.1-2.4)	3.5 (2.9-4.1)	2.7 (2.1-3.4)	1.7 (1.1-2.4)
Prodromal AD	Preclinical AD	3.8 (2.4-5.3)	2.1 (1.3-3)	1 (0.4-1.9)	4.4 (3.1-6.2)	1.8 (1.4-2.3)	0.7 (0.4-1.1)	2.6 (1.7-3.6)	1.3 (0.9-1.8)	0.6 (0.3-1.1)	3.2 (2.3-4.4)	1.6 (1.2-2.1)	0.7 (0.4-1.1)
	Prodromal AD	3.8 (2.9-4.5)	3.1 (2.6-3.6)	2.5 (1.7-3.2)	4.9 (4-5.7)	4.6 (4.1-5.1)	4.1 (3.4-4.8)	4.7 (4-5.4)	4.5 (4-5)	4.1 (3.5-4.8)	4.6 (4-5.3)	4.4 (3.9-4.9)	4 (3.4-4.7)
	Mild AD dementia	4.3 (3.6-4.7)	3.7 (3.2-4)	3 (2.2-3.4)	5.1 (4.4-5.7)	4 (3.6-4.2)	2.8 (2.4-3.1)	4.5 (4-4.8)	3.9 (3.5-4.2)	2.9 (2.5-3.3)	4.5 (4.1-4.9)	3.9 (3.5-4.2)	3 (2.5-3.4)
	Moderate AD dementia	5.2 (4.2-5.9)	4.3 (3.4-5)	3.2 (2.2-4)	4.5 (3.7-5.2)	3.6 (3.1-4.3)	2.7 (2-3.5)	5 (4.4-5.6)	3.9 (3.3-4.5)	2.7 (2.1-3.4)	4.9 (4.3-5.5)	3.9 (3.3-4.6)	2.7 (2.1-3.4)
Mild AD dementia	Mild AD dementia	4.7 (4-5.3)	4.1 (3.7-4.4)	3.4 (3-3.7)	6.5 (5.4-7.5)	4.8 (4.4-5.2)	3.4 (3.1-3.8)	4.9 (4.3-5.5)	4.3 (3.9-4.6)	3.5 (3.2-3.9)	5 (4.4-5.6)	4.3 (4-4.7)	3.6 (3.2-4)
	Moderate AD dementia	6.3 (5.4-7.2)	5.1 (4.4-5.9)	3.9 (3.2-4.9)	5.8 (4.5-7.2)	4.5 (3.9-5.2)	3.4 (2.8-4.2)	6 (5.2-6.8)	4.8 (4.3-5.4)	3.6 (3-4.4)	6 (5.2-6.8)	4.8 (4.2-5.5)	3.6 (2.9-4.4)
Moderate AD dementia	Moderate AD dementia	6.8 (5.6-7.9)	5.6 (4.8-6.6)	4.5 (3.7-5.7)	6.8 (4.7-9.2)	5.2 (4.2-6.2)	3.9 (3.3-4.8)	6.5 (5.4-7.6)	5.2 (4.6-5.9)	4.2 (3.4-5)	6.5 (5.5-7.6)	5.2 (4.6-6)	4.2 (3.4-5.1)

Moderate AD dementia = moderate to severe AD dementia. Model with age as covariate. *All estimates have overlapping confidence interval with confidence intervals based on the full dataset Table 2, model 2 in table B2.

Table B.7a Estimated difference in duration and p-values between setting

Starting stage	Duration, time in years (CI, 95%)	Age 60 Research vs clinical setting	p-value	Age 70 Research vs clinical setting	p-value	Age 80 Research vs clinical setting	p-value
Preclinical AD	Preclinical AD	10.08 (7.45,12.63)	<0.0001	7.7 (5.87,9.88)	<0.0001	5.62 (3.91,7.52)	<0.0001
	Prodromal AD	0.61 (-0.47,1.51)	0.2287	0.58 (-0.44,1.41)	0.2253	0.46 (-0.58,1.21)	0.3167
	Mild AD dementia	-0.14 (-0.92,0.59)	0.7351	-0.25 (-0.9,0.39)	0.4580	-0.38 (-0.98,0.15)	0.1797
	Moderate AD dementia	-2.62 (-3.58,-1.66)	<0.0001	-2.19 (-3.04,-1.4)	<0.0001	-1.70 (-2.48,-1.11)	<0.0001
Prodromal AD	Preclinical AD	1.6 (0.31,3.14)	0.0268	0.82 (0.21,1.46)	0.0099	0.39 (0.1,0.67)	0.0075
	Prodromal AD	1.5 (0.4,2.53)	0.0059	1.36 (0.4,2.22)	0.0035	1.14 (0.34,1.82)	0.0026
	Mild AD dementia	1.22 (0.36,2.16)	0.0079	0.97 (0.29,1.7)	0.0068	0.57 (0.1,1.17)	0.0385
	Moderate AD dementia	-1.86 (-2.95,-0.64)	0.0017	-1.55 (-2.59,-0.58)	0.0026	-1.25 (-2.06,-0.5)	0.0017
Mild AD dementia	Mild AD dementia	1.90 (0.89,3.05)	<0.0001	1.57 (0.75,2.48)	0.0001	1.13 (0.57,1.8)	0.0010
	Moderate AD dementia	-1.67 (-3.02,-0.18)	0.0208	-1.42 (-2.6,-0.22)	0.0196	-1.19 (-2.16,-0.23)	0.0161
Moderate AD dementia	Moderate AD dementia	-1.59 (-3.11,0.16)	0.0569	-1.33 (-2.66,0.14)	0.0635	-1.10 (-2.28,0.12)	0.0713

Moderate AD dementia = moderate to severe AD dementia. Model includes age as continues and setting as dichotomous covariates. Based on model 2 in table B.2. P-values based on confidence intervals of differences based on 500 simulation with the same seed. To interpret as positive is longer for research and negative is longer for the clinical setting.

Table B.7b Estimated duration by setting

Starting stage	Duration, time in years (CI, 95%)	Age 60		Age 70		Age 80	
	Setting	Research	Clinical	Research	Clinical	Research	Clinical
Preclinical AD	Preclinical AD	15.6 (12.9-17.5)	5.8 (4.1-7.6)	11 (9.5-12.6)	3.5 (2.5-5)	7.5 (5.8-9.4)	2.1 (1.4-3.3)
	Prodromal AD	4.4 (3.6-5)	3.8 (3.1-4.5)	4 (3.2-4.6)	3.4 (2.8-4.3)	3.5 (2.3-4.3)	3 (2.2-4)
	Mild AD dementia	3.9 (3.2-4.6)	4.1 (3.6-4.5)	3.3 (2.6-3.9)	3.5 (3.1-3.9)	2.3 (1.6-3)	2.7 (2.2-3.2)
	Moderate AD dementia	2.4 (1.8-3.1)	5.1 (4.3-6)	1.9 (1.4-2.6)	4.2 (3.5-5.2)	1.3 (0.8-1.9)	3.1 (2.4-4)
	Total duration	26 (23-28)	19 (17-20)	20 (18-22)	15 (13-16)	15 (12-17)	11 (10-12)
Prodromal AD	Preclinical AD	3.2 (2.2-4.6)	1.6 (1-2.5)	1.5 (1.1-2.1)	0.7 (0.4-1.2)	0.7 (0.4-1.1)	0.3 (0.1-0.6)
	Prodromal AD	5.3 (4.3-6.2)	3.8 (3-4.6)	4.7 (4.2-5.2)	3.4 (2.7-4.2)	4 (3.5-4.7)	2.9 (2.2-3.9)
	Mild AD dementia	5.6 (4.7-6.4)	4.4 (3.9-4.9)	4.8 (4.2-5.4)	3.8 (3.5-4.2)	3.5 (2.9-4.1)	3 (2.6-3.4)
	Moderate AD dementia	3.7 (2.9-4.7)	5.7 (4.9-6.6)	2.9 (2.3-3.7)	4.6 (3.9-5.5)	2 (1.5-2.7)	3.4 (2.7-4.4)
	Total duration	18 (16-19)	16 (14-17)	14 (13-15)	13 (12-14)	10 (9-11)	10 (9-11)
Mild AD dementia	Mild AD dementia	6.5 (5.4-7.6)	4.6 (4-5.3)	5.6 (4.8-6.5)	4 (3.6-4.3)	4.4 (3.7-5)	3.2 (2.9-3.6)
	Moderate AD dementia	4.6 (3.6-5.9)	6.4 (5.6-7.4)	3.7 (2.9-4.6)	5.3 (4.5-6.1)	2.7 (2.1-3.6)	4.1 (3.3-5.1)
	Total duration	11 (10-13)	11 (10-12)	9 (8-10)	9 (8-10)	7 (6-8)	7 (6-8)
Moderate AD dementia	Moderate AD dementia	5 (3.7-6.7)	6.8 (5.8-8.2)	4.1 (3.2-5.2)	5.6 (4.8-6.5)	3.3 (2.5-4.3)	4.6 (3.8-5.7)

Moderate AD dementia = moderate to severe AD dementia. Model includes age as continues and setting as dichotomous covariates. Based on model 2 in table B.2. P-values based on confidence intervals of differences based on 500 simulation with the same seed.

Table B.8 All six models with baseline transition rates and hazard ratios, sensitivity analysis in those with complete covariate data (= reduced sample size, n=1518)

Model 1 AGE

	Preclinical AD to prodromal AD	Preclinical AD to death	Prodromal AD to preclinical AD	Prodromal AD to mild dementia	Prodromal AD to death	Mild AD to moderate AD dementia	Mild AD dementia to death	Moderate AD dementia to death
Main analysis								
Transition rate, at age 70	0.083 (0.066,0.103)	0.002 (0.001,0.010)	0.049 (0.039, 0.062)	0.199 (0.176,0.223)	0.004 (0.001,0.011)	0.200 (0.181,0.220)	0.004 (0.001,0.014)	0.164 (0.140,0.191)
Age, per year increase	1.027 (1.001,1.053)	1.057 (0.897,1.245)	0.951 (0.923,0.979)	1.004 (0.990,1.018)	1.126 (1.027,1.240)	1.011 (1.0001,1.022)	1.163 (1.068,1.268)	1.024 (1.010,1.038)
Sample all variables (n=1518)								
Transition rate, at age 70	0.119 (0.091, 0.155)	0.002 (0.000, 0.017)	0.048 (0.037, 0.062)	0.185 (0.162, 0.211)	0.004 (0.002,0.012)	0.196 (0.171,0.225)	0.001 (0.000, 0.018)	0.178 (0.148,0.214)
Age, per year increase	1.012 (0.980,1.045)	1.104 (0.874,1.393)	0.945 (0.915,0.976)	1.004 (0.988,1.020)	1.115 (1.018,1.220)	0.990 (0.973,1.006)	1.258 (1.083,1.461)	1.030 (1.010,1.051)

	Preclinical AD to prodromal AD	Preclinical AD to death	Prodromal AD to preclinical AD	Prodromal AD to mild dementia	Prodromal AD to death	Mild AD to moderate AD dementia	Mild AD dementia to death	Moderate AD dementia to death
Main analysis								
Transition rate, at age 70	0.060 (0.046, 0.078)	0.003 (0.001,0.010)	0.0407 (0.0300,0.05)	0.1783 (0.1538,0.2068)	0.003 (0.001,0.011)	0.151 (0.125, 0.182)	0.005 (0.002, 0.015)	0.206 (0.1548, 0.274)
Age, per year increase	1.047 (1.020,1.073)	1.049 (0.894,1.230)	0.963 (0.933,0.994)	1.011 (0.996,1.027)	1.128 (1.024,1.243)	1.012 (1.001,1.022)	1.148 (1.065,1.238)	1.022 (1.008,1.036)
Clinic setting (ref=research setting)	4.832 (3.106,7.519)	-	2.132 (1.259,3.61)	1.450 (1.114,1.887)	-	1.446 (1.188,1.760)	-	0.750 (0.554,1.014)
Sample all variables (n=1518)								
Transition rate, at age 70	0.072 (0.050,0.103)	0.002 (0.000,0.018)	0.036 (0.026,0.051)	0.159 (0.135,0.189)	0.004 (0.002,0.012)	0.151 (0.117,0.196)	0.001 (0.000,0.076)	0.175 (0.144,0.211)
Age, per year increase	1.050 (1.016,1.085)	1.097 (0.867,1.388)	0.964 (0.930,0.999)	1.014 (0.997,1.032)	1.116 (1.019,1.222)	1.003 (0.982,1.025)	1.257 (0.995,1.588)	1.030 (1.009,1.052)
Clinic setting (ref=research setting)	4.278 (2.546,7.187)		2.384 (1.346, 4.223)	1.586 (1.191,2.111)		1.568 (1.088, 2.259)		0.493 (0.000,37651)

Model 3 AGE/SEX/SETTING

	Preclinical AD to prodromal AD	Preclinical AD to death	Prodromal AD to preclinical AD	Prodromal AD to mild dementia	Prodromal AD to death	Mild AD to moderate AD dementia	Mild AD dementia to death	Moderate AD dementia to death
Main analysis								
Transition rate, at age 70	0.0682 (0.049,0.094)	0.003 (0.001,0.010)	0.040 (0.027,0.058)	0.166 (0.138,0.199)	0.003 (0.001,0.011)	0.137 (0.111,0.168)	0.005 (0.001,0.022)	0.263 (0.193,0.357)
Age, per year increase	1.046 (1.020,1.074)	1.054 (0.903,1.231)	0.965 (0.934,0.997)	1.013 (0.998,1.029)	1.127 (1.020,1.245)	1.011 (1.001,1.023)	1.164 (1.067,1.227)	1.025 (1.010,1.040)
Female (ref=male)	0.769 (0.534,1.107)	-	1.028 (0.639,1.651)	1.154 (0.930,1.431)	-	1.237 (1.039,1.473)	0.446 (0.169,1.174)	0.6021 (0.456,0.795)
Clinic setting (ref=research setting)	4.403 (2.793,6.943)	-	1.975 (1.150,3.389)	1.477 (1.134,1.924)	-	1.410 (1.158,1.718)	-	0.771 (0.570,1.045)
Sample all variables (n=1518)								
Transition rate, at age 70	0.098 (0.064,0.150)	0.002 (0.000,0.018)	0.037 (0.024,0.056)	0.153 (0.124,0.188)	0.004 (0.001, 0.012)	0.142 (0.109, 0.186)	0.001 (0.000,0.045)	0.307 (0.205,0.460)
Age, per year increase	1.046 (1.012,1.082)	1.103 (0.883,1.378)	0.964 (0.930,0.9996)	1.015 (0.998,1.033)	1.118 (1.021,1.224)	1.006 (0.987,1.025)	1.268 (1.044,1.539)	1.013 (0.989,1.038)
Female (ref=male)	0.568 (0.365, 0.883)		0.947 (0.561, 1.599)	1.089 (0.851,1.393)		1.146 (0.881,1.491)	0.110 (0.000,31.177)	0.693 (0.486,0.990)
Clinic setting (ref=research setting)	4.125 (2.424,7.020)		2.476 (1.392,4.404)	1.597 (1.199,2.128)		1.605 (1.185,2.173)		0.581 (0.366,0.924)

	Preclinical AD to prodromal AD	Preclinical AD to death	Prodromal AD to preclinical AD	Prodromal AD to mild dementia	Prodromal AD to death	Mild AD to moderate AD dementia	Mild AD dementia to death	Moderate AD dementia to death
Main analysis								
Transition rate, at age 70	0.043 (0.032,0.062)	0.002 (0.001,0.009)	0.0427 (0.030,0.066)	0.133 (0.106,0.167)	0.004 (0.001,0.011)	0.196 (0.151,0.255)	0.001 (0.000,0.020)	0.193 (0.127,0.293)
Age, per year increase	1.061 (1.033,1.090)	1.0638 (0.906,1.249)	0.963 (0.932,0.996)	1.017 (1.001,1.033)	1.124 (1.025,1.232)	1.004 (0.987,1.021)	1.292 (1.086,1.538)	1.022 (1.0003,1.04)
APOE ε4 carrier (ref=non-carrier)	1.632 (1.106,2.408)	-	0.932 (0.566,1.534)	1.495 (1.178,1.897)	-	0.781 (0.608,1.003)	-	1.132 (0.796,1.611)
Clinic setting (ref=research setting)	4.501 (2.786,7.273)	-	1.890 (1.088,3.284)	1.444 (1.101,1.894)	-	1.481 (1.133,1.935)	-	0.704 (0.468,1.060)
Sample all variables (n=1518)								
Transition rate, at age 70	0.063 (0.041,0.098)	0.001 (0.000,0.019)	0.037 (0.022,0.060)	0.126 (0.097,0.162)	0.004 (0.001, 0.012)	0.185 (0.136,0.252)	0.001 (0.000,0.028)	0.226 (0.142,0.359)
Age, per year increase	1.053 (1.019,1.088)	1.097 (0.856,1.406)	0.964 (0.930,0.999)	1.019 (1.001,1.037)	1.118 (1.020,1.224)	1.004 (0.986,1.023)	1.303 (1.066,1.593)	1.016 (0.992,1.040)
APOE ε4 carrier (ref=non-carrier)	1.271 (0.804,2.009)		0.973 (0.563,1.683)	1.425 (1.095,1.855)		0.760 (0.577,1.002)		1.192 (0.820,1.732)
Clinic setting (ref=research setting)	4.087 (2.413,6.921)		2.417 (1.364,4.284)	1.546 (1.161,2.059)		1.588 (1.170,2.155)		0.592 (0.374,0.938)

	Preclinical AD to prodromal AD	Preclinical AD to death	Prodromal AD to preclinical AD	Prodromal AD to mild dementia	Prodromal AD to death	Mild AD to moderate AD dementia	Mild AD dementia to death	Moderate AD dementia to death
Main analysis								
Transition rate, at age 70	0.068 (0.046,0.099)	0.001 (0.000,0.022)	0.049 (0.033,0.071)	0.115 (0.092,0.145)	0.004 (0.001,0.012)	0.137 (0.099,0.189)	0.001 (0.0004,0.02)	0.284 (0.018,0.450)
Age, per year increase	1.035 (1.001,1.071)	1.073 (0.810,1.422)	0.973 (0.940,1.007)	1.011 (0.994,1.028)	1.112 (1.014,1.219)	1.003 (0.984,1.021)	1.274 (1.072,1.513)	1.016 (0.993,1.040)
Abnormal baseline CSF tau (ref=normal baseline CSF tau)	1.494 (0.949,2.352)	-	0.407 (0.234,0.709)	1.914 (1.481,2.475)	-	1.225 (0.901,1.664)	-	0.843 (0.557,1.276)
Clinic setting (ref=research setting)	3.166 (1.876,5.342)	-	2.811 (1.563,5.057)	1.332 (1.006,1.764)	-	1.513 (1.125,2.035)	-	0.606 (0.388,0.946)
Sample all variables (n=1518)								
Transition rate, at age 70	0.064 (0.044,0.095)	0.001 (0.000,0.020)	0.050 (0.034,0.072)	0.113 (0.090, 0.143)	0.004 (0.001,0.012)	0.134 (0.097,0.186)	0.001 (0.000,0.024)	0.295 (0185,0.469)
Age, per year increase	1.043 (1.008,1.079)	1.080 (0.817,1.428)	0.971 (0.938,1.006)	1.012 (0.994,1.030)	1.115 (1.014,1.226)	1.005 (0.986,1.024)	1.280 (1.072,1.529)	1.015 (0.991,1.039)
Abnormal baseline CSF tau (ref=normal baseline CSF tau)	1.430 (0.908,2.252)		0.387 (0.221,0.680)	1.905 (1.470,2.468)		1.187 (0.870,1.620)		0.832 (0.548,1.265)
Clinic setting (ref=research setting)	3.915 (2.281,6.720)		2.977 (1.643,5.393)	1.386 (1.038,1.850)		1.572 (1.158,2.134)		0.592 (0.373,0.940)

Model 6 subsample with all variables (n=1518) AGE/SEX/*APOE*/ TAU/ SETTING

	Preclinical AD to prodromal AD	Preclinical AD to death	Prodromal AD to preclinical AD	Prodromal AD to mild dementia	Prodromal AD to death	Mild AD to moderate AD dementia	Mild AD dementia to death	Moderate AD dementia to death
Transition rate, at age 70	0.079 (0.047,0.134)	0.001 (0.0001,0.02)	0.044 (0.025,0.077)	0.096 (0.071,0.130)	0.004 (0.001,0.012)	0.159 (0.109,0.231)	0.0006 (0.000,0.032)	0.302 (0.176, 0.531)
Age, per year increase	1.042 (1.005,1.080)	1.079 (0.825,1.410)	0.976 (0.941,1.013)	1.016 (0.998,1.035)	1.120 (1.017,1.234)	1.005 (0.986,1.024)	1.294 (1.053,1.589)	1.015 (0.991,1.039)
Female (ref=male)	0.562 (0.359,0.878)	-	1.072 (0.625,1.838)	0.997 (0.778,1.279)	-	1.120 (0.853,1.444)	-	0.700 (0.487,1.007)
APOE ε4 carrier (ref=non-carrier)	1.201 (0.756,1.909)	-	1.197 (0.681,2.105)	1.318 (1.010,1.720)	-	0.749 (0.568,0.988)	-	1.117 (0.766,1.628)
Abnormal baseline CSF tau (ref=normal baseline CSF tau)	1.470 (0.923,2.340)	-	0.358 (0.199,0.643)	1.846 (1.417,2.405)	-	1.189 (0.866,1.632)	-	0.928 (0.603,1.427)
Clinic setting (ref=research setting)	3.335 (1.884,5.905)		2.801 (1.522,5.157)	1.368 (1.022,1.830)	-	1.559 (1.1501,2.11)	-	0.587 (0.370,0.931)

Hazard ratios that are different from 1 in bold. Moderate = moderate to severe

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Chapter 2.2

Alzheimer Disease biomarkers may aid in the prognosis of MCI cases initially reverted to normal

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Abstract

OBJECTIVE: To identify potential predictors for outcome in individuals with mild cognitive impairment (MCI) who have reverted to normal cognition (NC).

METHODS: We selected individuals with MCI, who reverted at follow-up to NC, with follow-up after reversion from ADNI. Common clinical markers, AD biomarkers, and neurodegeneration imaging markers were used to compare MCI reverters based on subsequent clinical outcome (i.e. subsequent decline or stable reversion). For independent comparison, findings of the clinical Amsterdam Dementia Cohort are presented.

RESULTS: Seventy-seven (10%) out of 757 individuals with MCI reverted to NC and 61 individuals of these had follow-up data available. After 3.2±2.2 years 16 (24%) progressed to MCI, and 3 (5%) to dementia. Those who declined were older and had a higher amyloid PET burden and higher cerebrospinal fluid (CSF) tau levels.

CONCLUSION: In MCI reverters, abnormal biomarkers for AD pathology are associated with subsequent decline. AD biomarkers may aid in the prognosis of reverting MCI.

1 Introduction

Individuals with mild cognitive impairment (MCI) are at increased risk to develop dementia [1]. Yet, up to 25% of individuals with MCI revert to normal cognition (NC) [2, 3]. Although improved cognition seems to be a positive event, individuals reverting from MCI remain at increased risk to develop dementia compared to NC individuals [1, 4, 5]the Sydney Memory and Ageing Study. RESULTS While prevalence of MCI and different MCI subtypes remains relatively stable across all assessments, reversion from MCI and transitions between different MCI subtypes were common. Up to 46.5% of participants classified with MCI at baseline reverted at some point during follow-up. The majority (83.8%. Timely identification of individuals with a higher risk will increase prognostic certainty for patients and be useful for health care planning.

In individuals with NC and MCI, low memory function, abnormal biomarkers for Alzheimer Disease (AD), and neurodegeneration predict dementia [6, 7]. While MCI reverters deviate from the common clinical trajectory, the same disease processes may be underlying. Our aim was to investigate whether MCI reverters who subsequently showed clinical decline have more abnormal AD markers than MCI reverters who remain stable.

2 Methods

2.1 Participants

Data analyzed were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu, downloaded at 2017/8/9). From the individuals with at least two years clinical follow-up, we selected all individuals with prevalent and incident MCI reverting to NC with additional follow-up after reversion [8]. The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can measure progression to MCI and early AD. Next to the primary analyses in ADNI, we selected from the Amsterdam Dementia Cohort (ADC) all MCI reverters with follow-up after reversion. Similar clinical and biomarker assessments are presented for this small, independent clinical sample for illustration purposes only (for cohort and biomarker methods [9]).

2.2 Standard protocol approvals, registrations, and participant consents

All protocols were approved by an ethical review board and participants signed informed consent.

2.3 Clinical markers and *APOE*

All individuals had baseline data on age, sex and education. *APOE* genotype was dichotomized into ε4 carriers and non-carriers. Overall cognitive status was assessed by the MMSE, memory by the Rey Auditory Verbal Learning Test (RAVLT) immediate

(0-75) and delayed total recall (0-15), executive function by the Trial making test (TMT) A and B (seconds) and depressive symptoms by the Geriatric Depression Scale (GDS) (0-15). Subthreshold depression was classified as GDS>4 [10].

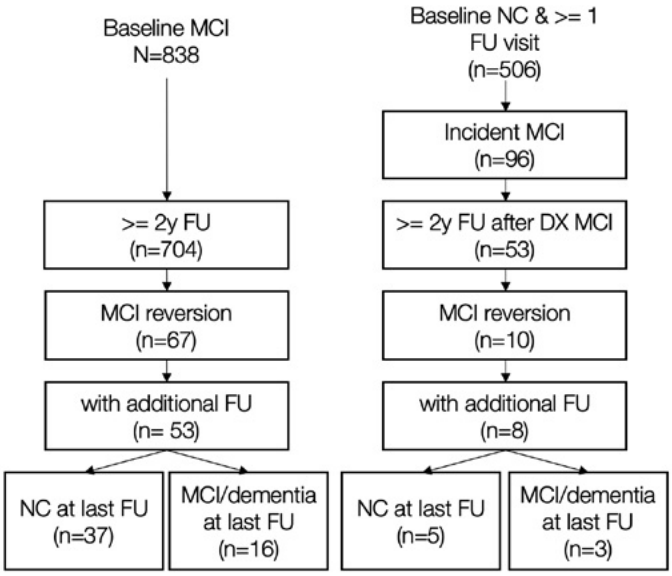

Figure 1 Flow diagram sample selection ADNI
N = number of individuals; MCI = Mild cognitive impairment; NC = cognitively normal; FU= follow-up visit; DX= diagnosis

2.4 Biomarkers of AD and neurodegeneration

We studied CSF amyloid beta 1-42 (Aβ1-42) and total tau (tTau) (Luminex in ADNI [11]; Innotest in ADC [12]), and amyloid PET (Florbetapir and PIB) as markers for AD pathology. PIB scans were harmonized to Florbetapir by: new value=PIB standard uptake value ratio (SUVr)*.67+.15[13]. For imaging markers of neurodegeneration, we studied FDG-PET, hippocampal volume (HV, UCSF in Freesurfer v4.4/v5.1), normalized to total intracranial volume, and white matter hyperintensity volume (WMH [14]2 and the primary goal of ADNI, the lifetime risk for stroke equals and may exceed the risk of AD in some circumstances 3. In addition, MRI evidence of asymptomatic cerebrovascular disease (CVD). Cut points for abnormality for dichotomized analysis in ADNI were: CSF Aβ1-42<192 pg/ml, CSF tTau>93pg/ml, amyloid PET SUVr>1.10, FDG-PET SUVr METAROI<1.21 and raw HV<6732 mm^3 ([11, 12, 15, 16] for procedures and processing). Data collected within one year before or after MCI diagnosis were included.

Table 1 MCI reverters with follow-up of ADNI and ADC

	ADNI MCI reverters				Amsterdam Dementia Cohort MCI reverters	
	Persistent normal cognition (n = 42)	Decline to MCI or dementia (n = 19)	p-value ADNI group comparison	p-value adjusted for age, sex, education, *APOE* ε4	Persistent normal cognition (n = 24)	Decline to MCI or dementia (n = 2)
Baseline characteristics						
Age, y	69 (8)	74 (8)	0.016	NA	65 (7)	71 (7)
Female, %	50%	26%	0.146	NA	29%	100%
Education, ADNI, y ADC, Verhage scale	17.2 (2.6)	16.3 (2.0)	0.095	NA	5 (1.4)	5 (1.4)
APOE E4 carrier, %	38%	32%	0.839	NA	46%	50%
Follow-up						
Total follow-up y, median (IQR)	4 (2.3)	5 (2.5)	0.109	NA	3.0 (1.8)	5.3 (1.6)
Time to reversion y, median (IQR)	1 (1.8)	2 (2)	0.462	NA	1.3 (1.0)	1.8 (0.7)
Follow-up after reversion y, median (IQR)	2 (1.8)	3 (2)	0.265	NA	1.4 (0.9)	3.6 (1.0)
Time to progression after reversion y, median (IQR)	NA	1 (1)	NA	NA	NA	1 (0)
N with > 1 reversion	4	2	>0.99	NA	2	1
Clinical						
MMSE	28.7 (1.4)	28.3 (1.8)	0.573	0.904	27.5 (1.6)	29
RAVLT immediate total recall	43 (11)	47 (12)	0.262	0.002	36 (10)	19
RAVLT delayed total recall	6.6 (4.2)	8.3 (4.6)	0.185	0.002	5.6 (1.6)	3
Trial making test A	31 (10)	34 (11)	0.496	0.700	38 (11)	44 (1)
Trial making test B	72 (24)	80 (31)	0.362	0.973	90 (36)	94 (30)
Geriatric depression scale (GDS)	1.1 (1)	1.6 (2)	0.138	0.018	3.7 (3)	3.5 (2)
GDS > 4, n (%)	2 (5%)	1 (5%)	>0.99	0.508	7 (32%)	1 (50%)

	ADNI MCI reverters				Amsterdam Dementia Cohort MCI reverters	
	Persistent normal cognition (n = 42)	Decline to MCI or dementia (n = 19)	p-value ADNI group comparison	p-value adjusted for age, sex, education, *APOE* ε4	Persistent normal cognition (n = 24)	Decline to MCI or dementia (n = 2)
AD biomarkers						
Amyloid PET, SUVR	1.08 (0.15)	1.21 (0.21)	0.026	0.016	-	-
Amyloid PET, n SUVR > 1.10 (%)	10 (30%)	9 (64%)	0.065	0.018	-	-
Luminex CSF Aβ1-42, pg/mL^	218 (45) ^	190 (65) ^	0.214	0.213	-	-
Innotest CSF Aβ1-42, pg/mL^	-	-			1047 (243)^	780 (5) ^
Abnormal CSF Aβ1-42, n (%)^	9 (31%)	5 (45%)	0.629	0.455	4 (20%)	2 (100%)
Luminex CSF total tau, pg/mL^	53 (17) ^	84 (42) ^	0.042	0.020	-	-
Innotest CSF total tau, pg/mL^	-	-			284 (140) ^	955 (24) ^
Abnormal CSF total tau, n (%)^	0 (0%)	3 (27%)	0.024	0.009	3 (15%)	2 (100%)
Imaging markers of neurodegeneration						
FDG PET METAROI, SUVR	1.34 (0.11)	1.27 (0.14)	0.051	0.458	-	-
FDG PET METAROI, SUVR < 1.21, n (%)	5 (13%)	6 (35%)	0.126	0.627	-	-
Hippocampus/Intracranial volume, cm^3	0.48 (0.07)	0.42 (0.09)	0.092	0.591	-	-
Hippocampus volume < 6673 mm^3, n (%)	6 (27%)	5 (56%)	0.280	0.731	-	-
White matter hyperintensities volume, cm^3	1.80 (2.69)	4.29 (6.24)	0.263	0.054	-	-

Data are mean (SD) unless otherwise specified; Bold = significant level < 0.05; Italic < 0.10; * if no biomarker data was available at the first MCI visit the data within 12 months was used. ^for ADNI: Luminex assay abnormality threshold: CSF Aβ1-42 <192 pg/mL, total tau >93 pg/mL; in ADC Innotest values corrected for upwards drift with abnormality thresholds CSF Aβ1-42 <813 pg/mL; total tau >375 pg/mL; Verhage scale range 1 to 7. MMSE=Mini-mental state examination. RAVLT=Rey auditory verbal learning test. Sample sizes in ADNI: Amyloid PET: n = 47; FDG PET: n = 55; MR hippocampal volumes n = 31; White matter hyperintensities: n = 58; CSF: n = 40. Sample sizes in Amsterdam Dementia Cohort: RAVLT: n=24; GDS: n=24; CSF: n =22.

2.5 Statistical analysis

MCI reverters with NC at last follow-up and MCI reverters with subsequent decline were compared on clinical and biomarkers using Chi-square, Wilcoxon and t-tests when appropriate. We report results unadjusted and adjusted for age, sex, education, and *APOE* ε4 genotype with univariate linear regression models, and scaling of continuous outcomes, to facilitate comparability of effects.

2.6 Data-sharing statement

Data used for this study are available from the corresponding author, upon reasonable request.

3 Results

In ADNI, 757 individuals with prevalent or incident MCI had been followed for at least two years (Figure 1). Of these, 77 (10%) reverted to NC, and 61 (79%) had additional follow-up available. After 3.2±2.2 years (mean±SD) 16 (24%) had converted to MCI, and 3 (5%) to dementia. One individual was excluded, due to missing data.

MCI reverters who showed subsequent clinical decline were on average 5 years older than reverters remaining NC, and had, adjusted for age, sex, education and *APOE*, higher and more often abnormal AD biomarkers (amyloid PET and CSF tTau), less impaired memory and higher GDS scores (Table 1/Figure 2). Follow-up after reversion seemed slightly shorter for stable MCI reverters (p=0.11). Repeating analyses including this covariate did not essentially changed the results (Table S1).

Post-hoc analyses further showed that biomarkers of MCI reverters were on average more similar to NC than non-reverting MCI, except for amyloid, which was more often abnormal in MCI reverters than in NC (Table S2). Still, MCI reverters showed higher clinical progression rates (110/1000 person-years) compared to baseline NC (52/1000 person-years, hazard ratio [95% CI] = 2.3 [1.4-4.0], p=0.002, Table S3/ Figure S1). The biomarker associations with progression were similar for NC and MCI reverters, whereas associations with progression and cognitive test scores were less consistent (Table S4/Figure S2).

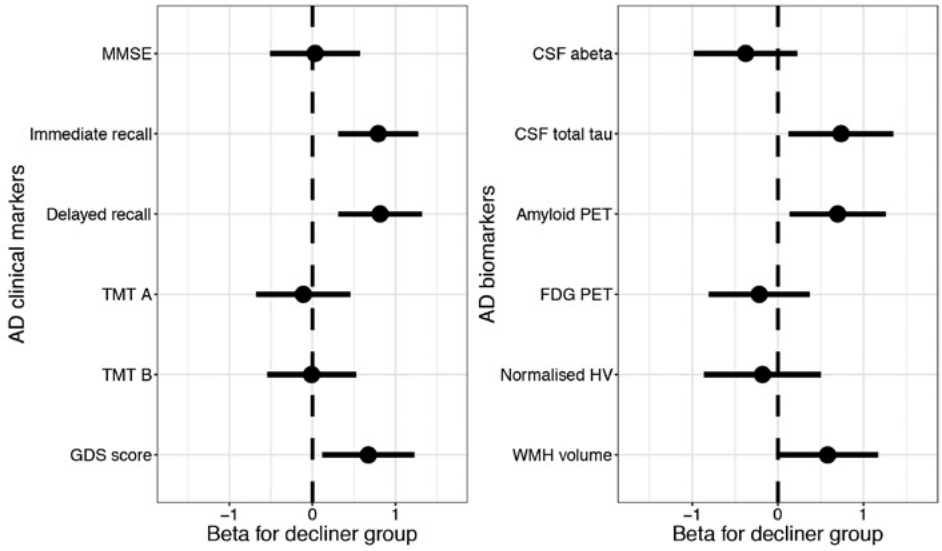

Figure 2 Standardized beta's AD clinical and biomarker for decliner group
Immediate and delayed recall of the RAVLT (Rey auditory verbal learning test); TMT = Trail making test; GDS = Geriatric depression scale; WMH = white matter hyperintensities; HV = hippocampal volume. Models were adjusted for age, sex, education and *APOE* ε4.

3.1 Outcome of MCI reverters in clinical ADC cohort

In the ADC, of 735 patients with MCI and a follow-up visit, 75 (10%) reverted to NC. Twenty-six (35%) patients had 1.6±0.8 years (mean±SD) follow-up available after reversion, after which 24 (92%) remained NC and 2 (8%) had dementia. Small group size precluded formal statistical testing. The two decliners had abnormal CSF Aβ1-42 and tTau (Table 1). The majority of individuals remaining NC had normal CSF Aβ1-42 (80%) and tTau (85%). Thirty-two percent of the stable reverters showed baseline subthreshold depression.

4 Discussion

Age and AD biomarkers are associated with decline in MCI patients who initially reverted to normal cognition. MCI reverters showed higher clinical progression rates than NC individuals, which is in line with previous reports [1, 4]. MCI reverters with subsequent decline had an increased amyloid PET burden and CSF tau compared to reverters remaining normal. Between amyloid markers, amyloid PET showed a significant association with the subsequent decline group in MCI reverters, while this association was significant for CSF $A\beta_{42}$ in NC. Although previous research suggests that CSF amyloid becomes abnormal before PET [17, 18], the findings are in line with other reports that this may not apply to all individuals [19, 20], which contributes to the

notion that CSF Aβ42 and amyloid PET may represent different AD-related processes.

An outstanding question is why individuals with underlying AD temporarily improved. Our results suggest that at baseline MCI reverters were more similar to NC than non-reverting MCI. Furthermore, biomarker values associated with subsequent decline were similar for reverting MCI and NC, while cognitive measures were less consistent. Possibly, reverters with decline received an MCI diagnosis very early in their clinical disease course, as their biomarker profiles was alike the non-reverting MCI. A modest improvement e.g., due to learning effects, resolving of (subthreshold) depressive symptoms or measurement error, may have contributed to reclassification as normal. Here we observed that when AD is present, such improvement is often not lasting.

Furthermore, it remains unclear as to why individuals who reverted and remained NC over time were initially diagnosed with MCI. Aside neurodegenerative diseases, depressive symptoms are a common cause of MCI. Low depressive symptoms scores in ADNI reflect inclusion criteria. In the ADC subthreshold depression was more common. Another possibility is that distress or insecurity led to a suboptimal performance. The question remains how to deal with the classification of these individuals in the context of AD disease progression research, when MCI is often regarded as an intermediate disease stage. A practical implementation could be to classify reverting MCI with normal biomarkers as NC. Alternatively, including stability of the diagnosis in the classification has been suggested [4].

A limitation of this study is the relatively short follow-up time, and so we cannot exclude the possibility that some individuals in the stable group may progress again. Compared to population-based studies, reversion rates in both cohorts were low [3]. Possibly, this reflects that clinicians will not easily reverse a known diagnosis. Reversion rates may even be lower, because we based reversion rates on individuals with MCI that met our inclusion criteria. Individuals with MCI excluded from these analyses as they were lost to follow-up were somewhat older and more cognitively impaired, which are characteristics that associate with decline [1] (Table S5). Although further replication in large population-based studies is necessary, our results suggest that AD biomarkers aid in the prognosis of MCI reverters, and could help to identify those with a good short term prognosis and those likely to decline again in the longer term.

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Declarations

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Supplemental data Chapter 2.2

Table S1 MCI reverters stable versus decliner adjusted for follow-up time after revision.

	ADNI MCI reverters
	p-value adjusted for age, sex, education, *APOE* ε4, and duration FU after reversion
Clinical	
MMSE	0.915
RAVLT immediate total recall	0.003
RAVLT delayed total recall	0.003
Trial making test A	0.817
Trial making test B	0.979
Geriatric depression scale (GDS)	0.023
GDS > 4, n (%)	0.540
AD biomarkers	
Amyloid PET, SUVR	0.017
Amyloid PET, n SUVR > 1.10 (%)	0.019
Luminex CSF $A\beta_{42}$, pg/mL	0.219
Abnormal CSF $A\beta_{42}$, n (%)	0.461
Luminex CSF total tau, pg/mL	0.020
Abnormal CSF total tau, n (%)	0.008
Imaging markers of neurodegeneration	
FDG PET METAROI, SUVR	0.980
FDG PET METAROI, SUVR < 1.21, n (%)	0.879
Hippocampus/Intracranial volume, cm^3	0.605
Hippocampus volume < 6673 mm^3, n (%)	0.743
White matter hyperintensities volume, cm^3	0.030

Table S2 Baseline NC and non-reverting MCI compared to baseline MCI reverters

	NC	MCI Non-reverting	MCI reverters	NC vs MCI reverters		Non-reverting MCI vs reverters	
	(n = 460)	(n = 637)	(n = 67)	p-value	Adjusted for age, sex, education, APOE ε4	p-value	Adjusted for age, sex, education, APOE ε4
Baseline characteristics							
Age, y	74 (6)	73 (7)	69 (8)	<0.001	-	<0.001	-
Female, %	51%	40%	43%	0.327	-	0.738	-
Education, y	16.4 (2.7)	15.9 (2.8)	16.8 (2.3)	0.306	-	0.018	-
APOE ε4 carrier, %	28%	51%	39%	0.102	-	0.089	-
Total follow-up, y	5 (3)	4 (2)	5 (2)	<0.001	-	<0.001	-
Follow-up after reversion, y (n=53)	-	-	3 (2)	-	-	-	-
Average % yearly progression to MCI or dementia	4.4%	-	9.8%	-	-	-	-
Average % yearly progression to dementia	1.2%	9.7%	1.5%	-	-	-	-
Clinical							
MMSE	29.1 (1.2)	27.5 (1.8)	28.7 (1.3)	0.025	0.012	<0.001	<0.001
RAVLT immediate total recall	45 (10)	34 (10)	43 (11)	0.282	0.006	<0.001	<0.001
RAVLT delayed total recall	7.6 (4)	3.6 (3.7)	7.1 (4)	0.368	0.068	<0.001	<0.001
Trail making test A	34 (12)	42 (19)	32 (10)	0.085	0.828	<0.001	0.003
Trail making test B	83 (40)	117 (66)	76 (23)	0.022	0.868	<0.001	<0.001
GDS	0.8 (1)	1.7 (1)	1.3 (1)	0.002	0.003	0.024	0.013
GDS>4	8 (2%)	30 (5%)	3 (4%)	0.312	0.328	>0.99	0.836

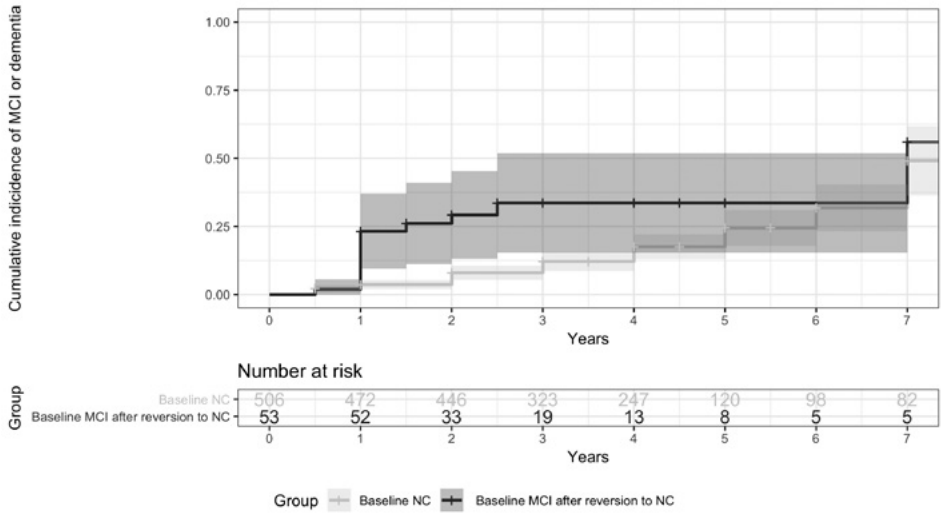
	NC	MCI Non-reverting	MCI reverters	NC vs MCI reverters		Non-reverting MCI vs reverters	
	(n = 460)	(n = 637)	(n = 67)	p-value	Adjusted for age, sex, education, APOE ε4	p-value	Adjusted for age, sex, education, APOE ε4
AD biomarkers							
Amyloid PET, SUVR	1.11 (0.18)	1.22 (0.22)	1.12 (0.16)	0.362	0.203	0.005	0.025
Amyloid PET SUVR > 1.11, %	87 (35%)	202 (58%)	22 (41%)	0.240	0.021	0.038	0.167
Luminex CSF $A\beta_{42}$, pg/mL^	204 (52)	168 (52)	206 (47)	0.708	0.934	<0.001	<0.001
Abnormal CSF $A\beta_{42}$, n (%)^	133 (40%)	327 (68%)	19 (37%)	0.801	0.950	<0.001	<0.001
Luminex CSF total tau, pg/mL^	67 (32)	92 (53)	62 (27)	0.203	0.892	<0.001	<0.001
Abnormal CSF total tau, n (%)^	63 (19%)	188 (39%)	3 (6%)	0.037	0.178	<0.001	<0.001
Imaging of neurodegeneration							
FDG PET METAROI, SUVR	1.31 (0.12)	1.24 (0.13)	1.32 (0.12)	0.535	0.575	<0.001	0.001
FDG PET METAROI, SUVR < 1.21, %	65 (19%)	195 (40%)	11 (17%)	0.929	0.594	0.001	<0.001
Hippocampus/ Intracranial volume, cm^3	0.46 (0.1)	0.39 (0.1)	0.48 (0.1)	0.214	0.918	<0.001	<0.001
Hippocampus volume < 6673 mm^3, %	132 (41%)	305 (72%)	9 (26%)	0.131	0.826	<0.001	<0.001
WMH volume, cm^3	3.5 (7.7)	4.0 (6.9)	2.6 (4.1)	0.791	0.925	0.487	0.333

All >=2yr FU after baseline visit. Baseline CN includes the CN with incident MCI and then reversion. MCI reverters includes all MCI reverters with MCI at the baseline visit, also those without additional FU, but not the incident MCI who reverted. Available sample: amyloid PET n=651, CSF n=865, FDG n=894, HV=779, WMH=1139.

Table S3 Hazard ratio's for progression of MCI reverters to MCI or dementia compared to NC

		HR [95% CI]	p-value
Model 1 (unadjusted)	NC vs MCI reverters	2.34 (1.38-3.99)	0.002
Model 2 (adjusted)	NC vs MCI reverters	2.30 (1.33-3.92)	0.003
	Age at baseline or reversion	1.04 (1.01-1.07)	0.010
	Sex - male	1.39 (0.95-2.04)	0.088
	Education	0.95 (0.89-1.02)	0.137
	APOE e4	1.59 (1.08-2.34)	0.019

Figure S1 Cumulative incidence of MCI or dementia in NC (green) compared to baseline MCI who reverted (orange).



Model 1 of table above. The groups include all baseline NC (n=506, progression n=101 (5 immediate to dementia) and MCI reverters (n=53, progression n=16) with follow-up visits. For the MCI only those with baseline MCI to avoid overlapping subjects. Progression to MCI or dementia for NC was 52 per 1000 person-years, and for the MCI reverters 110 per 1000 person-years.

Table S4 ADNI Predictors of progression in baseline NC compared to the MCI reverters

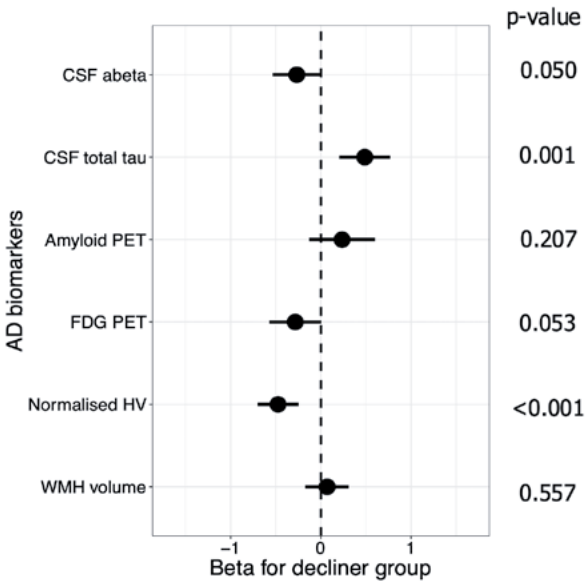
	Baseline CN stable vs progression			MCI reverters (copy table 1)		
	Persistent normal cognition (n = 377)	Decline to MCI or dementia (n = 83)	CN stable vs CN decline p-value^	Persistent normal cognition (n = 42)	Decline to MCI or dementia (n = 19)	p-value^
Baseline characteristics						
Age, y	74 (6)	76 (5)	<0.001	69 (8)	74 (8)	0.016
Female, %	52%	45%	0.281	50%	26%	0.146
Education, y	16.5 (2.7)	16.1 (2.5)	0.216	17.2 (2.6)	16.3 (2.0)	0.095
APOE ε4 carrier, %	27%	34%	0.270	38%	32%	0.839
Total follow-up y, median (IQR)	4 (2)	5.5 (7)	<0.001	4 (2.3)	5 (2.5)	0.109
Clinical						
MMSE	29.1 (1.2)	29.1 (1.1)	0.262	28.7 (1.4)	28.3 (1.8)	0.904
RAVLT immediate total recall	46 (10)	41 (10)	0.003	43 (11)	47 (12)	0.002
RAVLT delayed total recall	8 (3.8)	6 (3.9)	0.001	6.6 (4.2)	8.3 (4.6)	0.002
Trail making test A	34 (11)	38 (13)	0.042	31 (10)	34 (11)	0.700
Trail making test B	82 (40)	88 (37)	0.998	72 (24)	80 (31)	0.973
GDS	0.8 (1)	1.1 (1)	0.009	1.1 (1)	1.6 (2)	0.018
GDS>4	5 (1%)	3 (4%)	0.085	2 (5%)	1 (5%)	0.508
AD biomarkers						
Amyloid PET, SUVR	1.11 (0.17)	1.17 (0.21)	0.207	1.08 (0.15)	1.21 (0.21)	0.016
Amyloid PET SUVR > 1.11, %	70 (32%)	16 (55%)	0.070	10 (30%)	9 (64%)	0.018
Luminex CSF $A\beta_{42}$, pg/mL^	207 (51)	188 (51)	0.050	218 (45)	190 (65)	0.213
Abnormal CSF $A\beta_{42}$, n (%)^	105 (38%)	30 (54%)	0.094	9 (31%)	5 (45%)	0.455
Luminex CSF total tau, pg/mL^	64 (30)	82 (35)	0.001	53 (17)	84 (42)	0.020
Abnormal CSF total tau, n (%)^	42 (15%)	21 (38%)	<0.001	0 (0%)	3 (27%)	0.009
Imaging markers of neurodegeneration						
FDG PET METAROI, SUVR	1.32 (0.11)	1.28 (0.13)	0.053	1.34 (0.11)	1.27 (0.14)	0.458
FDG PET METAROI, SUVR < 1.21, %	46 (16%)	18 (31%)	0.053	5 (13%)	6 (35%)	0.627
Hippocampus/Intracranial volume, cm^3	0.47 (0.07)	0.43 (0.05)	<0.001	0.48 (0.07)	0.42 (0.09)	0.591
Hippocampus volume < 6673 mm^3, %	92 (36%)	40 (61%)	0.002	6 (27%)	5 (56%)	0.731
WMH volume, cm^3	3.32 (6.47)	4.28 (11.95)	0.577	1.80 (2.69)	4.29 (6.24)	0.054

All baseline NC with >= 2y follow-up (n=460). ^Clinical, AD and imaging markers comparisons are adjusted for age, sex, education, and *APOE* ε4.

Table S5 Included and excluded MCI individuals based at least 2 years of follow-up time

	Included sample of MCI individuals	Excluded MCI individuals	Included vs Excluded	
	(n = 757)	(n = 177)	p-value	Adjusted for age, sex, education, *APOE* ε4
Baseline characteristics				
Age, y	73 (8)	76 (8)	<0.001	-
Female, %	41%	41%	0.985	-
Education, y	16.0 (2.8)	15.6 (2.9)	0.149	-
APOE ε4 carrier, %	48%	54%	0.209	-
Clinical				
MMSE	27.7 (1.8)	27.4 (2.0)	0.090	0.426
RAVLT immediate total recall	35 (11)	32 (11)	0.002	0.058
RAVLT delayed total recall	4.0 (3.9)	3 .2(3.5)	0.006	0.090
Trail making test A	40 (18)	44 (22)	0.027	0.014
Trail making test B	112 (63)	131 (71)	0.001	0.014
GDS	1.6 (1)	1.9 (2)	0.187	0.033
AD biomarkers				
Amyloid PET, SUVR	1.21 (0.22)	1.25 (0.24)	0.159	0.788
Amyloid PET, n SUVR > 1.10 (%)	55%	64%	0.106	0.646
Luminex CSF $A\beta_{42}$, pg/mL	172 (54)	165 (49)	0.196	0.734
Abnormal CSF $A\beta_{42}$, n (%)	352 (65%)	65 (71%)	0.262	0.814
Luminex CSF total tau, pg/mL	89 (51)	98 (59)	0.155	0.612
Abnormal CSF total tau, n (%)	229 (36%)	57 (37%)	0.824	0.464
Imaging markers of neurodegeneration				
FDG PET METAROI, SUVR	1.25 (0.13)	1.20 (0.14)	0.001	0.007
FDG PET METAROI, SUVR < 1.21, n (%)	217 (38%)	53 (49%)	0.053	0.246
Hippocampus/Intracranial volume, cm^3	0.40 (0.08)	0.40 (0.08)	0.943	0.479
Hippocampus volume < 6673 mm^3, n (%)	328 (69%)	91 (72%)	0.529	0.909
White matter hyperintensities volume, cm^3	3.94 (7.04)	3.96 (6.77)	0.306	0.598

Figure S2 Biomarkers beta's for progression group vs stable group in normal cognition.



WMH = white matter hyperintensities; HV = hippocampal volume. Univariate analysis.

3

Chapter 3

Recruitment for Alzheimer disease research

Chapter 3.1

European Prevention of Alzheimer Dementia (EPAD) Registry: recruitment and pre-screening approach for a longitudinal cohort and prevention trials.

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Abstract

BACKGROUND: It is a challenge to find participants for Alzheimer Disease (AD) prevention trials within a short period of time. The European Prevention of Alzheimer Dementia (EPAD) Registry aims to facilitate recruitment by preselecting subjects from ongoing cohort studies. This paper introduces this novel approach.
METHODS: A virtual registry, with access to risk factors and biomarkers for AD through minimal datasets of ongoing cohort studies, was set up.
RESULTS: To date, ten cohorts have been included in the EPAD Registry. Around 2500 participants have been selected, using variables associated with the risk for AD. Of these, 15% were already recruited in the EPAD longitudinal cohort study, which serves as a trial readiness cohort.
DISCUSSION: This study demonstrates that a virtual registry can be used for the preselection of participants for AD studies.

1 Introduction

1.1 Finding participants for secondary prevention of Alzheimer Disease

Finding participants for Alzheimer Disease (AD) trials is challenging [1]. This is particularly the case for studies with prodromal or preclinical AD participants, because these persons may not seek care for their problems and are unaware of the presence of amyloid pathology. An increasing number of AD trials aims to delay the onset of dementia in prodromal and preclinical AD. Traditional ad-hoc recruitment strategies, such as advertising in newspapers, result in a costly, labor-intensive and long recruitment process with many screen failures. Novel pre-selection and patient recruitment strategies are warranted. Online registries or the use of existing data sources may help to facilitate recruitment [2]. EPAD Registry makes use of existing data sources for recruitment in a virtual registry in order to speed up recruitment, reduce recruitment efforts, and reduce screen failures.

1.2 EPAD Registry as part of the EPAD project

The EPAD Registry is part of the European Prevention of Alzheimer Dementia (IMI-EPAD) project. This project is meant to create a platform for AD secondary prevention trials and to improve the understanding of the development of AD, by setting up the EPAD Registry, EPAD longitudinal cohort study (EPAD-LCS) and EPAD proof-of-concept trials (EPAD-PoC) [3]. The EPAD Registry was set-up to find participants without dementia for the EPAD-LCS. In the EPAD-LCS participants undergo longitudinal assessments of cerebral spinal fluid (CSF), blood, MRI, AD risk factors and cognition. The primary outcome is the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). The EPAD-LCS serves as the trial readiness cohort for EPAD-PoCs. The first EPAD-PoC is planned to start in 2018.

The EPAD Registry preselects participants from ongoing cohort studies and uses data from these cohorts for prescreening. The EPAD Registry involves several steps (figure 1). First the collaboration with an ongoing cohort study representative is established. Second a minimal dataset from the ongoing cohort study is created that can be queried in a software tool, called PREPAD. Potential participants are identified and invited for the EPAD-LCS by the EPAD study team. The results of these efforts are monitored.

2 Methods

2.1 Selection of and engagement with ongoing cohort studies

Cohorts that are selected for the EPAD Registry fulfil the following criteria: they are willing to provide participants for the EPAD-LCS, include participants without dementia over the age of 50, collected data suitable for prescreening, and have consent to contact their participants about the EPAD-LCS. In return the ongoing cohort studies will receive the data collected within the EPAD project for their participants. When interest is expressed by a cohort representative, cohort characteristics are collected online either in the European Medical Information Framework (EMIF) or Dementia Platform UK (DPUK) catalogues [4, 5]. Legal contracts are developed to cover interactions between cohorts and EPAD. These contracts cover the use of the virtual registry for EPAD purposes and receiving the EPAD data of their own participants.

2.2 Minimal dataset and PREPAD query platform

Each cohort is asked to provide a minimal dataset of variables that can be used to preselect participants with an increased risk for AD. The variables comprise age, gender, education, *apolipoprotein E* ε4 (*APOE* ε4) genotype, family history of dementia, diagnosis of cognitive disorder, CSF biomarkers, MRI hippocampal atrophy, memory test scores, and baseline and longitudinal minimal mental state examination (MMSE) scores. At least 4 of the above variables are required. The minimal dataset of each cohort is harmonized. Cohort specific harmonisation rules are run with every update. Cohort representatives were supported by a small EPAD Registry workgroup consisting of software developers and AD-researchers. AD-researchers used mock files from each of the cohorts to define harmonisation rules and shared those with the developers supporting the harmonisation. The minimal dataset is uploaded on a regular basis to the PREPAD software tool. PREPAD was developed to search these minimal datasets. It was adapted from an existing data discovery platform to allow for querying federated datasets and allow complex queries [6]. To ensure participants remain anonymous in the EPAD Registry, a software called 'Deridiom' was created that generates 'Derivative IDs' (derIDs). Deridiom converts cohort local identification numbers to derIDs.

Searching participants with PREPAD involves a number of steps. First an algorithm is defined that aims to identify participants, according to the needs of the EPAD-LCS and EPAD-PoC. The algorithm is tailored for each cohort allowing

selection of participants in different risk-stages for AD. An algorithm can for example be a decreased memory score and age over 65. The search results in a list of derIDs. This list is provided to a cohort representative who converts derIDs into local IDs and selects participants to invite for EPAD-LCS screening.

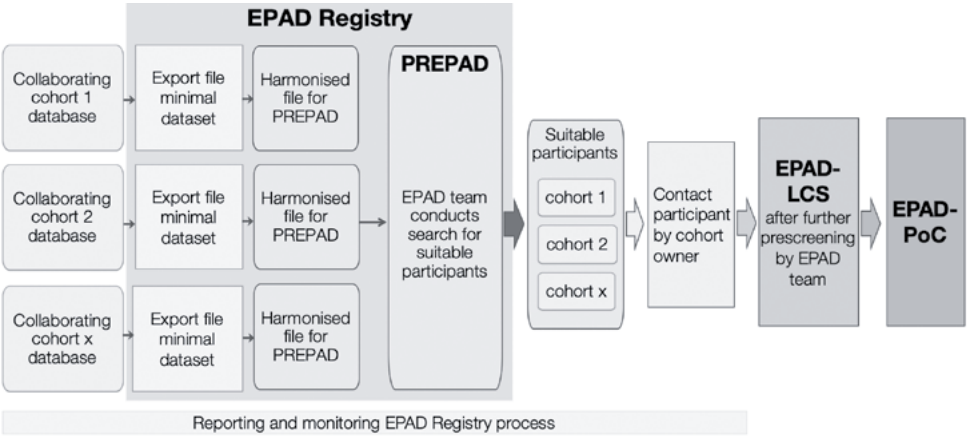

Figure 1 Schematic overview of data and participant flow in EPAD.
Abbreviations: EPAD-LCS = EPAD longitudinal cohort study; EPAD-PoC = EPAD proof-of-concept trials

2.3 From EPAD Registry to EPAD-LCS and measuring recruitment rate

After participants have been selected via PREPAD, cohort representatives use local additional pre-screening information to decide whether a participant should be invited for EPAD-LCS screening. Next, a cohort representative approaches a potential participant. Dependent on local preferences and legislation, an opt-in letter is sent or a phone call is made to invite participants to one of the EPAD centres where screening activities and EPAD-LCS procedures will be conducted by the EPAD-LCS team. During the first contact by EPAD with a potential participant, usually by phone, in- and exclusion criteria are checked, such as the availability of a study partner. At each step, summary counts are collected of the number of participants in the process and predefined reasons for pre-screen failure such as contra-indication, no contact possible, in other study, unspecified reason, no interest of the participant, and prefer to invite later.

3 Results

3.1 Cohorts

Twenty cohorts representatives from 8 countries completed the first step, the questionnaire about cohort characteristics, each of them giving access to 100 to 500,000 potential participants. Ten cohorts from France, Italy, the Netherlands,

Sweden, Spain, Switzerland and the UK are currently formally signed-up to PREPAD, providing access to 17,500 potential participants aged over 50 and without dementia [7-13]. Incentives for cohorts to participate were: acquiring follow-up and biomarker measures in a subset of the cohort, scientific involvement in EPAD project, and providing clinical trial access for participants. In Table 1a the distribution according to diagnosis and age is presented. The variables available in each of the cohorts are shown in Table 1b. Memory clinic cohorts often have amyloid data available, and almost all cohorts have information on the *APOE* ε4 status. All cohorts have at least one parameter on cognitive status.

3.2 Recruitment for EPAD-LCS via EPAD Registry

As of the first of June 2017, 2433 participants of the EPAD Registry were pre-selected, of whom 75% were invited for EPAD-LCS screening by the team of the collaborating cohorts. The main reason for not contacting a participant was a known exclusion criterion. Most cohorts chose to contact participants by phone. Thus far around 15% of the subjects selected were suitable and agreed to undergo EPAD-LCS screening. This percentage may increase as more of the participants in the EPAD Registry will be considered for EPAD-LCS screening. Reasons of potential participants not entering EPAD-LCS screening are variable and partly dependent on collaborating cohort type. We will monitor uptake prospectively and report on this in detail once we have sufficient data available.

Table 1 Number of participants of existing cohorts in the EPAD Registry June 2017 by age group

Age	CN	SCI	MCI	Total
50-64	9,065	511	83	9,659
65-79	5,160	265	219	5,644
>= 80	845	9	182	1,036
All	15,070	785	484	16,339

Abbreviations: CN, cognitively normal; MCI, mild cognitive impairment, SCI, subjective cognitive impairment. NOTE. The 2433 participants already selected are not included in the table.

Table 2 Prescreening data available on participants in existing cohorts of EPAD Registry June 2017

	Population based			Memory clinic							
Cohort characteristics	Generation Scotland [8]	Pilot Amsterdam Registry	PREVENT [7]	ALFA [10]	GAP [13]	GEDOC [12]	ADC [9]	French Trial Registry [2]	ARWIBO [11]	Epinettes	Total
Country	UK	NL	UK	Spain	Spain	Sweden	NL	France	Italy	SW	NA
N over age of 50*	12,600	500	200	2,400	400	150	400	200	1,700	200	18,750
Population	Sample of general population	Online registration of volunteers	High percentage off-spring AD patients	High percentage off-spring AD patients	Advertisement recruited	Consecutive patients	Consecutive patients	Consecutive patients	Consecutive patients	Consecutive patients of a 1year period	NA
Relevant in- and exclusion criteria	Select additional family member	None	No cognitive decline due to other causes	No cognitive decline due to other causes, study partner	No cognitive decline due to other causes	No cognitive decline due to other causes	No cognitive decline due to other causes	Consent for Registration for EPAD-Registry	None	None	NA
Age ranges at inclusion	18+	18+	45-65	45-74	45-65	50+	50+	50+	50+	50+	NA
General and demographics											
Diagnosis											
CN	√	√	√	√	√	-	-	√	√	√	8
SCI	-	√	-	√	√	√	√	√	√	√	8
MCI and/or MCI due to AD	-	√	-	-	√	√	√	√	√	√	7
Dementia	√	√	√	-	√	√	-	-	√	√	7
Demographics	√	√	√	√	√	√	√	√	√	√	10
Visit dates	√	√	√	√	√	√	√	√	√	√	10

Risk factors and biomarkers and cognitive tests											
APOE ε4	√	s	√	√	√	√	√	-	√	√	9
Family history of dementia	√	√	√	√	√	√	√	√	√	√	10
CSF biomarkers	-	-	-	P	√	√	√	-	√	√	5
Hippocampal atrophy	-	-	-	√	√	√	√	-	√	-	5
MMSE score	-	s	-	L	√	√	√ L	√	√	√	8
Memory test	WLM	Muistikko	Cognito	MBT	FCSRT	RAVLT	RAVLT	FCSRT	Babcock	FCSRT	10

Abbreviations: AD, Alzheimer disease; ADC, Amsterdam Dementia cohort; ALFA, for Alzheimer and Families project; *APOE* 34, apolipoprotein E 34; Babcock, Babcock short story memory test; CN, cognitively normal; Cognito, computerized test battery with narrative memory test and associative memory test; CSF, cerebrospinal fluid; EPAD, European Prevention of Alzheimer's Dementia; FCRST, Free Recall Selective Reminding Test (Grober-Buschke); GAP, Gipuzkoa Alzheimer Project; GEDOC, Clinical dementia research database Stockholm; Hippocampal atrophy, Medial temporal lobe score or volume corrected for intracranial volume; MBT, memory binding test; MCI, mild cognitive impairment; MMSE, minimal mental state examination; Muistikko, a web-based cognitive test battery; NL, The Netherlands; P, pending; PREVENT, cohort for mid-life biomarkers of late-onset AD; RALVT, Rey Auditory Verbal Learning Task; s, subset; SCI, subjective cognitive impairment; SW, Switzerland; UK, United Kingdom; WLM, Weschler logical memory test. NOTE. N* aged 50+, round to 50. NOTE. √, available; -, not available; √ L, 2
or more results available.

4 Discussion

The EPAD Registry provides a novel recruitment strategy. It is sufficiently flexible as we can adapt the screening algorithms to the type of data collected in a cohort and the type of participants needed for future EPAD-PoCs.-The collaborating cohorts have different levels of information and draw on different populations. We chose not to define strict criteria for collaboration, but instead to use the data that are available within the cohorts. This approach leads to collaboration with more cohorts than would have been possible otherwise. However, a limitation is that risk estimates vary over cohorts. The efficacy of the approach will be monitored and reported on in the future. This includes the recruitment rate and the characteristics of population recruited, which can then be compared to other strategies. It may well be that this approach is particularly effective for specific populations of volunteers of patients. The adaptation of existing software led to a fast implementation. Plans for further development of the EPAD Registry functions entail extending PREPAD with other risk factors and inclusion of information on exclusion criteria. We also intend further automation of the harmonization process.

Our approach for recruitment and prescreening differs from those used in other studies and trials aimed at preventing disease progression in preclinical AD. Examples are the Early trial with a BACE-inhibitor from Janssen and another BACE inhibitor trial, the A4 study, that stepwise screen individuals from over 65 years old found via advertisements or a website. Additionally for the Early trial, persons between 60 and 64 that have an additional risk factor, being a positive family history for AD or being an *APOE* ε4 allele carrier can be screened [14, 15]. The API-*APOE*4 trial from the Banner Institute pioneers with *APOE* genotyping as a screening method to find suitable trial participants [16]. The MOPEAD project is set up to formally test different recruitment and pre-screening strategies, including an online memory screening and recruitment via a diabetes mellitus outpatient clinic [17]. Another approach is to let potential participants register online individually. Registrants provide prescreening information, after which the platform matches them to ongoing studies. The Brain Health Registry is a leading example of this. All studies mentioned are ongoing and have not reported yet on the recruitment and the screen failure rate. When the EPAD-PoCs have started, the EPAD Registry approach can be evaluated in terms of trial participation. Combining insights from these various approaches has the potential to greatly improve our understanding of the best ways to find participants for preclinical AD trials in the near future. Our approach could be adapted for other projects in the AD field and beyond, or to find participants within projects, if a minimal dataset for prescreening is available.

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Declarations

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Chapter 3.2

Prescreening for European Prevention of Alzheimer Dementia (EPAD) Trial-Ready Cohort: Impact of AD risk factors and recruitment settings

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Abstract

BACKGROUND: Recruitment is often a bottleneck in secondary prevention trials in Alzheimer disease (AD). Furthermore, screen-failure rates in these trials are typically high due to relatively low prevalence of AD pathology in individuals without dementia, especially among cognitively unimpaired. Prescreening on AD risk factors may facilitate recruitment, but the efficiency will depend on how these factors link to participation rates and AD pathology. We investigated whether common AD-related factors predict trial-ready cohort participation and amyloid status across different pre-screen settings.

METHODS: We monitored the prescreening in 4 cohorts linked to the European Prevention of Alzheimer Dementia (EPAD) Registry (n=16,877; mean±SD age=64±8 years). These included a clinical cohort, a research in-person cohort, a research online cohort, and a population-based cohort. Individuals were asked to participate in the EPAD longitudinal cohort study (EPAD-LCS), which serves as a trial-ready cohort for secondary prevention trials. Amyloid positivity was measured in cerebrospinal fluid as part of the EPAD-LCS assessment. We calculated participation rates and numbers needed-to-prescreen (NNPS) per participant that was amyloid-positive.

We tested if age, sex, education level, *APOE* status, family history for dementia, memory complaints or memory scores, previously collected in these cohorts, could predict participation and amyloid status.

RESULTS: 2,595 participants were contacted for participation in the EPAD-LCS. Participation rates varied by setting between 3% and 59%. The NNPS were 6.9 (clinical cohort), 7.5 (research in-person cohort), 8.4 (research online cohort), and 88.5 (population-based cohort). Participation in the EPAD-LCS (n=413 (16%)) was associated with lower age (odds ratio (OR) age = 0.97 [0.95-0.99]), high education (OR=1.64 [1.23-2.17]), male sex (OR=1.56 [1.19-2.04]), and positive family history of dementia (OR=1.66 [1.19-2.31]). Among participants in the EPAD-LCS, amyloid positivity (33%) was associated with higher age (OR=1.06 [1.02-1.10]) and *APOE* ε4 allele carriership (OR=2.99 [1.81-4.94]). These results were similar across prescreen settings.

CONCLUSIONS: Numbers needed-to-prescreen varied greatly between settings. Understanding how common AD risk factors link to study participation and amyloid positivity is informative for recruitment strategy of studies on secondary prevention of AD.

1 Background

Recruitment of participants for secondary prevention trials in Alzheimer Disease (AD) is challenging, which can cause substantial delays in study completion [1, 2]. The target population for these types of clinical trials typically comprises of individuals without signs of dementia, and with evidence of amyloid pathology [3]. Clinical trial screening of these mildly symptomatic or asymptomatic participants is accompanied by large numbers of screen failures [1]. The solution may be to introduce low-burden prescreening steps, which would limit the screening efforts to individuals with an increased prospect of enrolment into the study [4-7]. However, there is little empirical evidence on prescreening for secondary prevention trials and whether the efficacy depends on recruitment setting [8-11].

The European Prevention of Alzheimer Dementia (EPAD) Registry was set up as a virtual registry from existing cohorts [12]. The purpose was to enable recruitment and preselection of individuals for participation in the EPAD longitudinal cohort study (EPAD-LCS) [13], which also serves as a trial-ready cohort for the EPAD secondary prevention trials [14]. Data on several AD-related factors were available in these existing cohorts, including age, sex, education, *APOE* genotype, family history of dementia, subjective cognitive decline (SCD), and memory tests, as well as on common exclusion criteria. Furthermore, unlike in most trials, where a participant contacts a site following advertisements, in EPAD, researchers invited participants from the cohorts in the EPAD Registry into the EPAD-LCS. This approach allowed for investigation of how AD risk factors related to the participation rate, an important consideration for the feasibility assessment of

recruitment strategies. The recruitment settings linked to the registry include memory clinics, online and in-person brain research cohorts, and population-based cohorts, thereby offering the opportunity to compare them. We assessed participation rates across different recruitment settings, and provide a number needed-to-prescreen (NNPS) to identify one eligible and amyloid-positive individual. We also tested the AD-related factors as predictors for participation in the EPAD-LCS and for amyloid positivity.

2. Methods

2.1 Population

The analysis included participants from the first four cohorts that were linked to the EPAD Registry. The French Trial Registry in Toulouse selected patients referred by GPs and self-referral from memory clinics [15]. Inclusion criteria were: interest in clinical trials, available study partner and no obvious exclusion criteria for clinical trials. Data from 195 participants without dementia, with visits between July 2016 and February 2018, had been linked to the EPAD Registry. The ALFA Study included cognitively unimpaired individuals who expressed interest in participating in AD research and data of 2,595 participants aged over 50 years, with first visits in 2013 and 2014, were linked to the EPAD Registry [16]. Generation Scotland (GS) was a population-based study which collected data between 2006 and 2011 in Scotland on randomly drawn individuals with a relative to co-enrol [17]. Its aim was to create a resource of human biological samples and information for medical research, and data on 13,681 participants aged over 50 years, without a known diagnosis of dementia, were linked to the EPAD Registry. The pilot 'hersenonderzoek.nl' (pilotHO.nl) was a web-based registry with the aim of recruiting people from the general public for brain research and ran from Sept 2016 to Sept 2017 when the final version of the registry was launched. This pilot registry had 412 participants, age over 50 years and without a self-reported diagnosis of dementia, linked to the EPAD Registry.

2.2 EPAD Registry selection and prescreening process

The enrolment process for the EPAD-LCS consisted of 4 steps. In step 1, participants were preselected from the 4 cohorts using algorithms in the EPAD Registry online tool [18], based on different combinations of age, sex, diagnosis of mild cognitive impairment (MCI), *APOE* genotype, SCD, memory test scores, and/or family history for dementia, available in the parent cohort (Table 1). Flexible algorithms were tailored to each of the cohorts, and adjusted if the number of individuals meeting the algorithmic criteria was low. The algorithms selected individuals older than 50 years across an AD dementia risk spectrum [13]. These included those with low and medium risk for AD to reach the recruitment targets for the study, as well as to avoid AD risk status disclosure by invitation. In step 2, the cohorts' investigators checked eligibility of selected individuals, using data from their databases. These criteria included the EPAD in-

and exclusion criteria, which involve absence of disorders that could interfere with trial participation, absence of dementia, and openness to potentially participate in intervention studies and receive disclosure [13]. In three of the cohorts, preselected individuals were then approached by telephone for participation. The population-based cohort GS sent an opt-in letter. In step 3, the EPAD sites performed a telephone screen to check eligibility amongst those who expressed interest in participating. Prescreen failures during the first 3 steps were categorized as: 'matching an exclusion criterion', 'no interest in participation in the study', 'not returning the opt-in letter', 'other reason, not specified' [12]. In step 4, participants visited a site and enrolled in the EPAD-LCS for a screening/baseline visit, after which eligibility was confirmed and amyloid status was determined [13].

2.3 Data collected as part of the EPAD-LCS

From the EPAD-LCS baseline visit we used, clinical information, i.e., the CDR sum of boxes (CDR-SOB) and Mini-Mental State Examination (MMSE); structural MR imaging visual rating scales, i.e., the medial-temporal atrophy scale (MTA) mean score and Fazekas deep score of white matter hyperintensities. From the cerebrospinal fluid (CSF) analysis, we used Elecsys $A\beta_{42}$, total tau, and phosphorylated tau values, and from the blood analyses, for some participants, *APOE* ε4 genotype. For a full description of the EPAD-LCS protocols, we refer to [13].

2.4 Predictors

The predictors as collected in the cohorts linked to the Registry were: age, sex, education level (low to normal or high), *APOE* genotype (ε4 non-carrier or carrier), presence of family history for dementia, presence of SCD, and a low score on a delayed recall memory test (z-score < -1.28, details on definitions of variables Supplement, legend Table 1). All cohorts had data available on demographics. SCD data was present in all cohorts, except GS. *APOE* genotype was available in the ALFA Study, GS, and a subset of pilotHO.nl. Family history and memory test scores were available for all participants of the ALFA Study and GS, and for the majority in the Toulouse Registry and pilotHO.nl. The definitions of the predictors were as follows: high education was 14 years or more in Toulouse Registry, the ALFA Study, and GS, and in pilotHO.nl a score of 6 or more on the Verhage scale, equivalent to college or university level [19]. Subjective cognitive decline: presence of memory complaints in the absence of impairment on cognitive tests (Toulouse Registry); a positive answer on the question whether the participant memory had complaints (ALFA study), a positive answer on the questions whether the participant memory had complaints and worries about their memory (pilotHO). Low memory delayed recall z-score < -1.28 on the FCSRT delayed recall (Toulouse), the memory binding test (ALFA study), the Wechsler logical memory - delayed recall (GS), and the Muistikko-test (pilotHO).

2.5 Outcomes

The first outcome measure was enrolment into the EPAD-LCS, indicating participation in a screening/baseline visit. The second outcome was amyloid positivity, defined as CSF $A\beta_{42}$ below 1098 pg/mL [20-22], for participants who completed and passed the eligibility checks of the EPAD-LCS screening visit.

2.6 Statistical analysis

Participation rate was defined as the percentage of individuals who underwent the EPAD-LCS screening visit out of the individuals approached for participation in the EPAD-LCS. The NNPS was defined as the ratio between the number of individuals contacted for participation and the number of individuals that passed baseline visit classified as amyloid positive. The number needed-to-screen (NNS) was the ratio between the number of individuals with baseline data and the number of individuals that passed screening visit who were classified as amyloid positive. To test the association between AD risk factors (predictors) and participation into the EPAD-LCS, and among those enrolled, between AD risk factors and amyloid positivity, we applied univariate logistic mixed models with a random term for cohort and fixed term for the predictor. Age was centered at 65. Explorative analyses included analyses stratified now by cohort using univariate logistic regression models. Additionally, as a second step, all significant predictors for either of the two outcomes were combined in two final multivariate models to summarize the results. Statistical analyses were performed in R version 3.4.2, using packages 'lme4' and 'lmerTest' [23, 24].

3 Results

The four cohorts linked to the EPAD Registry included 16,877 participants. The participants were on average 64 (SD=8) years old and 39% were male, and expected amyloid positivity was calculated to be 19% based on a published meta-analysis [4] (Table 1). Figure 1 and Table 2 describe the recruitment flow of participants to enrolment and amyloid measurement in the EPAD-LCS between May 2016 and March 2018. Table 3 presents clinical, imaging and CSF markers of the EPAD-LCS baseline visit for participants recruited from each of the cohorts, stratified by amyloid status.

From the EPAD Registry, 3009 individuals were preselected for participation in the EPAD-LCS and 2,595 individuals were contacted, of whom 413 (16%) agreed to participate and were eligible for the EPAD-LCS screening visit. To prevent contacting individuals matching exclusion criteria for the EPAD-LCS, most cohorts conducted a database check. This was most efficient in the Toulouse registry (100%). Of individuals with exclusion criteria in the ALFA Study 75% (110/147), and in pilotHO.nl 55% (24/53) were found during the database check. Participation rate varied by setting; in the Toulouse Registry it was 59%, in the ALFA Study 56%, in GS 3%, and in pilotHO.nl 46%. The primary reasons for not participating were not returning the opt-in leaflet (67%), no interest (16%), and other reasons (13%). Of the 324 participants who had passed the eligibility checks during EPAD-LCS screening visit and had their amyloid

status available, 107 (33%) participants were amyloid positive. The total number of amyloid-positive individuals was similar between cohorts (Toulouse Registry n=23, ALFA Study n=36, GS n=22, pilotHO.nl n=26). However, the NNPS to find one eligible amyloid-positive participant varied; in the Toulouse Registry it was 6.9, in the ALFA Study 7.5, in GS 88.5, and in pilotHO.nl 8.4. Among individuals enrolled in the EPAD-LCS, the NNS in order to find one amyloid-positive individual passing the screening visit was between 3.0 and 3.8 in all settings (Table 2).

Table 1 Baseline available data and characteristics of cohorts

	Toulouse Registry	ALFA	Generation Scotland	pilotHO.nl
Setting	Memory clinic	In-person research cohort	Population-based	Online research cohort
N	195	2,589	13,681	412
Age, y	68 (7)	60 (6)	64 (9)	65 (9)
Male, n (%)	56 (29%)	962 (37%)	5399 (39%)	155 (38%)
Highly educated, n (%) (n= 15239)[*]	97 (60%)	1,225 (47%)	4,860 (40%)	313 (77%)
APOE ε4 genotype, n (%) (n= 16185)	NA	872 (34%)	3,695 (28%)	84 (31%)
Family history for dementia, n (%) (n= 16844)	131 (71%)	2,470 (95%)	1,386 (10%)	193 (50%)
Subjective cognitive decline, n (%) (n=3175)[^]	151 (83%)	312 (12%)	NA	81 (20%)
% low memory, n (%) (n= 16420)[$]	17 (15%)	242 (9%)	1,684 (12%)	20 (9%)
Diagnosed with MCI, n (%)[#]	13 (7%)	0	3 (0%)	4 (1%)
Estimated amyloid-positive individuals based on [4], taking into account age-bins, n (%)	~40 (22%)	~430 (17%)	~2680 (20%)	~80 (20%)

Legend: [*] high education: Toulouse Registry: >=14 years; ALFA Study:>=14 years; GS:>=14 years; pilotHO.nl: >=6 on the Verhage scale. [^] SCD: Toulouse Registry: physician diagnosis and MCI patients excluded; ALFA Study: memory complaints question; pilotHO.nl: questions on memory complaints with worries; [$] Low memory delayed recall z-score < -1.28: Toulouse Registry: FCSRT delayed recall, normalised by formula (score-11)/2, at raw score cut-off < 9; ALFA Study: memory binding test, normalised to sample, at raw score cut-off <18; GS: Wechsler logical memory - delayed recall was normalized, at raw score cut-off <9; pilotHO.nl: online Muistikko-test, normalized to sample, at raw score cut-off <9. [#] MCI: Toulouse Registry: physician diagnosis; pilotHO.nl: self-report.

Table 2 Recruitment flow from EPAD Registry by recruitment setting

	Cohorts				Total
	Toulouse Registry	ALFA Study	Generation Scotland	pilotHO.nl	
Setting	Memory clinic	In-person research cohort	Population-based	Online research cohort	
Step 1 Selection by PREPAD tool	169	618	1,947	275	3,009
Step 2 Not eligible	11	347	1	55	414
• Exclusion criterion	10	110	1	29	150
• Other	1	237	0	26	264
Selected for step 3	158	271	1,946	220	2,595
Step 3 Not eligible	65	119	1,879	119	2,182
• No interest	64	24	178	83	349
• No response to letter	NA	NA	1,470	NA	1,470
• Exclusion criterion	0	37	12	24	73
• Other	1	58	219	12	290
Eligible, selected for step 4	93	152	67	101	413
• % from step 2	56%	25%	3%	37%	14%
• % from step 3	59%	56%	3%	46%	16%
Step 4 EPAD-LCS screening visit	70	137	67	88	362
Eligible & CSF A1-42 analyzed	64	124	61	75	324
• CSF A1-42 < 1098 pg/mL(positivity)	23 (36%)	36 (29%)	22 (36%)	26 (35%)	107 (33%)
Number needed-to-screen	3.0	3.8	3.0	3.4	3.4
Number needed-to-prescreen	6.9	7.5	88.5	8.5	24.3

Legend: Number of individuals unless otherwise specified. EPAD-LCS v500 is the currently available data, quality checked at data lock. N=51 EPAD screening visit details not yet available. N=5 CSF results missing. N=32 screen failure: 11x other disease/incidental findings/CDR>=1, 18x procedures not possible, 3xinvestigator decision/no reason provided/no contact possible.

Table 3 Included participants in EPAD Longitudinal cohort study per recruitment setting

	Toulouse Registry		ALFA Study		Generation Scotland		pilotHO.nl	
	CSF Aβ +ve	CSF Aβ normal	CSF Aβ +ve	CSF Aβ normal	CSF Aβ +ve	CSF Aβ normal	CSF Aβ +ve	CSF Aβ normal
n	23	41	36	88	22	39	26	49
Age, y	71 (5)	67 (8)^	64 (6)	64 (5)	71 (3)	67 (5)#	68 (6)	66 (7)
Male, n (%)	4 (17%)	17 (41%)	23 (64%)	41 (47%)	14 (61%)	23 (57%)	14 (52%)	19 (37%)
MMSE (30-0)	28.0 (2.1)	28.8 (1.7)	28.6 (1.1)	28.7 (1.6)	28.1 (1.6)	28.8 (1.4)	28.4 (1.5)	29.1 (1.3)
CDR-SOB (0-18)	0.74 (0.7)	0.34 (0.5)^	0.10 (0.3)	0.05 (0.2)	0.15 (0.3)	0.06 (0.2)	0.06 (0.2)	0.02 (0.1)
CSF $A\beta_{42}$, pg/mL	756 (195)	1613 (361)#	823 (191)	1696 (519)#	748 (251)	1769 (411)#	846 (217)	1788 (443)#
CSF pTau, pg/mL	29 (15)	18 (4.9)$	21 (15)	17 (7)	19 (9)	21 (12)	21 (10)	17 (5)
CSF tTau, pg/mL	305 (125)	210 (53)$	223 (132)	209 (79)	211 (81)	249 (115)*	240 (101)	206 (58)
MTA (0-4)	0.4 (0.5)	0.2 (0.4)	0.2 (0.4)	0.1 (0.3)	0.4 (0.6)	0.1 (0.4)	0.2 (0.4)	0.2 (0.4)
Fazekas (0-3)	1.1 (0.7)	0.8 (0.7)	1 (0.6)	0.8 (0.6)	1.1 (0.9)	0.7 (0.7)^	0.9 (0.8)	0.9 (0.7)

Legend: pTau = phosphorylated tau. tTau = total tau. MTA = medial temporal lobe atrophy. Mean (SD) unless otherwise specified. *one outlier at tTau 792 and p-tau 81. Undetectably low p-tau and tTau was set at the detection border of 8 and 80 respectively, abeta 1-42 was extrapolated. Raw $p<0.05$ = ^; $p<0.01$ = $, $p<0.001$ = #.

Table 4 Univariate logistic regression for enrolment and CSF $A\beta_{42}$ positivity in whole sample and stratified by recruitment setting

	Total		Toulouse Registry		ALFA Study		Generation Scotland		pilotHO.nl	
Sample size	n=2,595	n=324	n=158	n=64	n=271	n=124	n=1,947	n=61	n=220	n=75
Outcome	Enrolment*	CSF Aβ +ve^	Enrolment*	CSF Aβ +ve^	Enrolment*	CSF Aβ +ve^	Enrolment*	CSF Aβ +ve^	Enrolment*	CSF Aβ +ve^
Aged over 70 Years Old	0.97	1.06	0.99	1.10	0.99	1.01	0.97	1.23	0.96	1.03
	(0.95-0.99)	(1.02-1.10)	(0.94-1.03)	(1.01-1.20)	(0.95-1.03)	(0.94-1.08)	(0.93-1.01)	(1.08-1.45)	(0.93-1.00)	(0.97-1.11)
Male	1.56	1.28	1.17	0.30	2.03	2.03	1.81	1.35	1.13	2.01
	(1.19-2.04)	(0.81-2.04)	(0.58-2.42)	(0.08-0.96)	(1.24-3.35)	(0.92-4.60)	(1.11-3.01)	(0.47-4.08)	(0.66-1.94)	(0.77-5.36)
Highly Educated	1.64	0.89	1.44	0.72	1.42	1.10	2.20	0.66	1.33	0.75
	(1.23-2.17)	(0.56-1.42)	(0.69-2.98)	(0.25-2.13)	(0.87-2.31)	(0.50-2.39)	(1.34-3.59)	(0.22-1.88)	(0.67-2.67)	(0.24-2.51)
APOE ε4 Genotype	0.95	2.99	NA	6.42	0.68	1.72	1.37	7.20	0.92	3.34
	(0.70-1.28)	(1.81-4.94)		(1.93-24.1)	(0.41-1.10)	(0.79-3.86)	(0.84-2.25)	(2.2-28.77)	(0.49-1.72)	(1.22-9.48)
Family history of Dementia	1.66	1.58	1.04	0.95	1.12	NA#	2.95	2.90	1.27	1.94
	(1.19-2.31)	(0.83-3.00)	(0.50-2.15)	(0.31-2.98)	(0.38-3.23)		(1.73-4.91)	(0.97-8.96)	(0.73-2.22)	(0.68-6.09)
Subjective Cognitive Decline	0.86	1.51	0.29	2.93	0.79	1.15	NA	NA	1.16	1.73
	(0.58-1.27)	(0.88-2.61)	(0.09-0.76)	(0.67-20.6)	(0.41-1.55)	(0.38-3.22)			(0.62-2.15)	(0.62-4.79)
Low Memory Score	0.84	1.47	0.63	18.90	0.95	1.29	0.78	0.95	0.91	0.58
	(0.60-1.17)	(0.82-2.61)	(0.21-1.87)	(2.87-377)	(0.56-1.64)	(0.55-2.96)	(0.44-1.31)	(0.28-3.02)	(0.29-2.83)	(0.03-4.98)

Odds ratio (95% CI); Bold is significant p<0.05. * = Odds ratio for participating baseline/ screening visit after invitation; ^ = Odds ratio for amyloid positivity among those included in EPAD-LCS ; # = infinite, not possible to calculate a value.

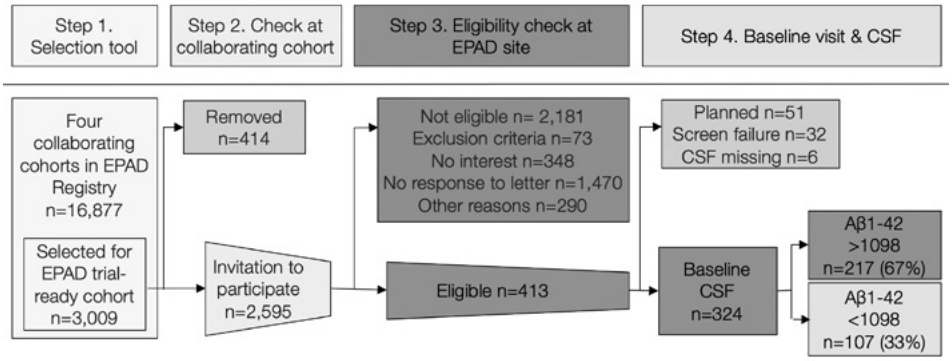

Figure 1 Prescreening to enrolment: flow from EPAD Registry to EPAD trial-ready cohort
Legend: CSF = cerebrospinal fluid; EPAD = European Prevention of Alzheimer Dementia.

3.1 Predictors for participation rate

The AD risk factors that were univariately associated with participation in the EPAD-LCS, for all cohorts combined, were lower age (odds ratio (OR): age=0.97 [0.95-0.99]), high education level (OR=1.64 [1.23-2.17]), male sex (OR=1.56 [1.19-2.04]) and family history of dementia (OR=1.66 [1.19-2.31], Table 4, for AUCs Table S2). In single cohorts, participation rates in the Toulouse Registry were predicted by SCD (OR=0.29; [0.09-0.76]), in the ALFA Study by male sex (OR=2.03 [1.24-3.35]), in GS by male sex (OR=1.81 [1.11-3.01]), high education (OR=2.20 [1.34-3.59], and family history (OR=2.95 [1.73-4.91], and in pilotHO.nl by age (OR=0.96 [0.93-1.00]). As a next step, we combined the predictor variables age, sex, education, family history, and *APOE* in a multivariate model (Figure 2, Supplement Table S1 and S3). Study enrolment was still associated with age, sex, education and family history (n with all variables = 2322).

3.2 Predictors for amyloid positivity

Among all individuals enrolled in EPAD-LCS, amyloid positivity was univariately predicted by older age (OR= 1.06 [1.02-1.10]) and carrying an *APOE* ε4 allele (OR=2.99 [1.81-4.94]) (Table 4, for AUCs Table S2). In individual cohorts, amyloid positivity in the Toulouse Registry was predicted by higher age (OR=1.10 [1.01-1.20]), gender (male OR=0.30 [0.08-0.96]), *APOE* ε4 (OR=6.42 [1.93-24.1]), and low memory (OR=18.90 [2.87-377], in the ALFA Study by none, in GS by higher age (OR=1.23 [1.08-1.45]) and *APOE* ε4 (OR=7.20 [2.20-28.77]), and in pilotHO.nl by *APOE* ε4 (OR=3.34 [1.22-9.48]). In the multivariate model, including predictor variables age, sex, education, family history, and *APOE*, amyloid status was predicted by age, *APOE* ε4, and weakly by family history (p=0.03, n with all variables = 322, Figure 2, Supplement Table S1 and S3).

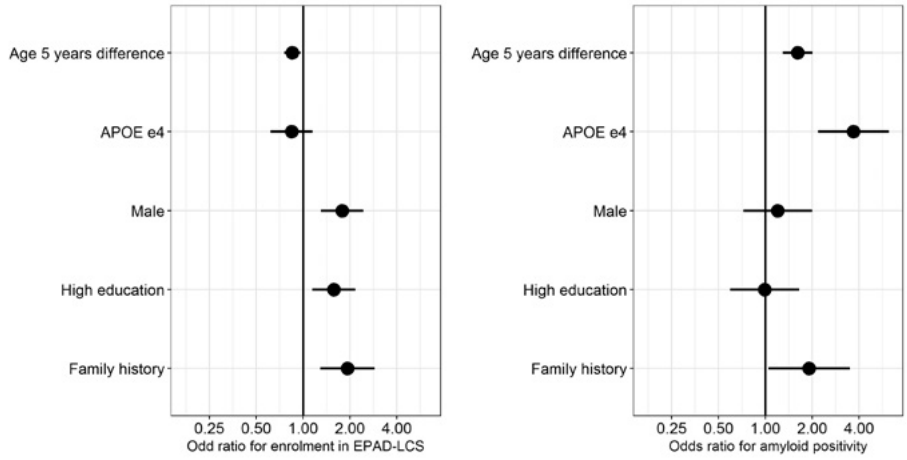

Figure 2 Multivariate model for enrolment and amyloid positivity
Legend: EPAD-LCS = EPAD longitudinal cohort study (trial-ready cohort). *APOE* = Apolipoprotein E genotype.

4 Discussion

Across settings, participation rates varied, while predictors for participation into the trial-ready cohort and amyloid positivity were comparable. Among those contacted for participation, enrolment was higher for individuals who were younger, more educated, males or had a family history of dementia, while amyloid positivity in the trial-ready cohort was only associated with being older and carrying an *APOE* ε4 allele.

The NNPS to find one amyloid-positive eligible participant in the population-based Generation Scotland study was ten times higher than for those cohorts focused on brain disorders, which may be explained by their willingness to take part in an AD study [25]. Generation Scotland study visits have been completed, and the time between the last Generation Scotland study visit and EPAD recruitment was also longer than for the other cohorts. In addition, an opt-in letter was sent to Generation Scotland participants, while other cohorts contacted individuals by telephone, which may have lowered the response [26]. Moreover, the EPAD study site was at a travel time of 1-3 hours from the recruitment region. Finally, the cohorts from the other settings excluded persons with known exclusion criteria beforehand based on data from their cohort database, which may have decreased later stage prescreen failures. Still, the number of participants recruited of the large population-based Generation Scotland cohort were comparable to the bespoke cohorts, suggesting that there is scope and willingness within these type of cohorts to participate in dementia related intervention studies.

Lower participation at older ages, and higher participation for both highly educated participants and those with a family history of dementia is in line with studies with dementia patients and online registers [9, 10, 27-29]. Barriers for older individuals to participate may include morbidities, difficulties to travel, and not having an study partner. The higher participation rate of males was unexpected,

as many research studies have lower male than female participation [9, 10, 30, 31].

The predictors for amyloid positivity, i.e., age and *APOE*, were as expected and in line with previous studies, including an EPAD-LCS full dataset analysis [4, 6, 32, 33]. Low memory scores, in contrast, were only a significant predictor for amyloid positivity in the memory clinic cohort and the presence of SCD did not predict amyloid positivity in our sample. As low memory scores low memory scores were the best predictor for amyloid positivity in the memory clinic setting, memory tests may form a useful prescreen in this situation. An explanation for the discrepancy with previously reported associations of these factors with amyloid status, could be the non-standardized test data, and could possibly show better predictive effects with the use of tailored sensitive tests and questionnaires [9, 11, 32, 34-37]

. The prevalence of amyloid positivity in those enrolled in the EPAD-LCS was 33%. This prevalence was enriched around 1.5 times compared to the estimated prevalence in the whole cohorts based on a meta-analysis of prevalence in cognitively normal individuals [4]. The limited increase in prevalence of amyloid positivity could be explained by the fact that the variables available for prescreening each have a modest predictive accuracy for amyloid positivity [4, 6]. Another explanation is that low- and intermediate-risk individuals were selected from the cohorts in order to prevent risk disclosure by invitation and to have sufficient enrolment in the EPAD-LCS.

An advantage of our approach compared to other recruitment strategies such as media campaigns advertisement is that the use of existing data helped to exclude individuals with known exclusion criteria for secondary prevention trials. However, no direct comparison of efficiency relative to other prescreening strategies (e.g. advertising) could be made. A disadvantage of our approach is that consent to re-contact needs to be present in the cohorts and some costs are involved in the prescreening. In addition, cohorts become depleted, as shown for the smaller cohorts in our study. Future projects could involve direct comparisons between recruitment strategies and focus on cost and effort monitoring and comparison. Another important factor when recruiting from collaborating studies, as well as in the gathering of a 'trial ready cohort' is the aspect of time and cohort maintenance costs of both the recruitment cohorts and EPAD-LCS, but substantial. As AD is a progressive disorder, the time between testing in a parent cohort and time of selection may be important. Future work on the EPAD-LCS and similar projects needs to optimize the costs and efforts of maintaining a trial ready cohort. This should also involve monitoring the rate at which individuals become ineligible over time, for example because they develop comorbidities that are exclusion criteria.

A limitation is that the analyses were done with the risk factors available in each cohort, such that not all risk factors were available in all cohorts for all individuals. Also, the use of the available data and adaptation to local standard procedures meant that there was variability in the operationalization of variables. Secondly, algorithms for preselection in the EPAD Registry tool included predictor variables of the current study. Still, that is unlikely to influence the association between each of the risk factors and

participation rate as multivariate models yielded similar results. Additionally, cohorts were different from each other in more than one factor, such as sample size, population characteristics and communication style. Therefore differences in recruitment rate may be explained by several factors. Despite the differences, participation rate was associated with similar AD risk factors across cohorts. Finally, we have now studied the participation in a trial-ready cohort, but enrolment into an actual clinical trial might give different results, depending on study-specific in- and exclusion criteria and trial design [38]. Strengths of our study are the prospective prescreening and the large sample in which amyloid-testing was performed.

Our comparison of common AD risk factors for their association with participation rate and amyloid positivity has several implications for prescreening strategies for secondary prevention trials aimed at individuals with amyloid pathology. Age was a relatively strong predictor for amyloid positivity. However, we also showed that elderly individuals were less likely to participate in the study, which would limit the prescreening efficiency of age for amyloid positivity. Therefore, addressing barriers for older individuals to participate could increase recruitment of eligible participants [29, 39]. Carrying an *APOE* ε4 allele was also a strong predictor of amyloid status but, as published before, the disadvantage is that around 40% of amyloid positive individuals are *APOE* ε4 non-carriers [40]. The prevalence of *APOE* ε4 positivity is around 20-30% and this may therefore not be optimal for prescreening in a small cohort. Disclosure of genotype could also be an issue [10, 41]. These limitations may be overcome by using a family history for dementia as a pre-screener. The advantage of this risk factor is the association with a greater enrolment rate, but the disadvantage is that its association with amyloid positivity is weak and the prevalence in the general population low. Subtle memory decline or concerns were not a useful prescreen for amyloid status in our study, but more specific tests or questionnaires may perform better [11, 42, 43]. A promising alternative may be blood tests for amyloid [5, 44, 45]. With a sensitive threshold, such a test has the advantage to more effectively prescreen relatively younger individuals, who often comprise a large part of a registry population and are more likely to participate, but have a low prevalence of amyloid pathology.

5 Conclusions

We found that enrolment rates show major differences between cohorts, although predictors for participation were similar. The provided NNPS to find one eligible amyloid-positive participant are indicators that future recruitment strategies can relate to. The findings highlight considerations of clinical trial investigators, balancing a gain in the ease of recruitment with potentially reducing the generalizability of the trial. Measures to increase efficiency for recruitment for secondary prevention trials may include using prospective registries with continuous enrolment of participants, adding a prescreening step with sensitive measures, such as a blood test, and addressing barriers for older and lower-educated individuals to participate.

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Declarations

Ethics approval and consent to participate: The EPAD-LCS (www.clinicaltrials.gov: NCT02804789) and the other cohorts were approved by the ethical review board in each country in which the study was performed, and all participants gave informed consent. The cohort studies had consent to contact participants for other research projects.

Consent for publication: Not applicable.

Availability of data and materials: The EPAD data used in this analysis will be made available on an open-access platform in due course. (www.ep-ad.org for updates).

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Supplemental data Chapter 3.2

Table S1 Multivariate logistic regression for enrolment and CSF Aβ1-42 positivity in whole sample

	Enrolment			CSF Aβ1-42 positivity		
Sample size		N=2322			N=322	
Outcome	Univariate	Multivariate	Multivariate p-values	Univariate	Multivariate	Multivariate p-values
Age years	0.97 (0.95-0.99)	0.97 (0.95-0.99)	0.011	1.06 (1.02-1.10)	1.10 (1.05- 1.15)	<0.001
APOE ε4 genotype	0.95 (0.70-1.28)	0.85 (0.62-1.15)	0.291	2.99 (1.81-4.94)	3.69 (2.18-6.24)	<0.001
Male	1.56 (1.19-2.04)	1.79 (1.31-2.45)	<0.001	1.28 (0.81-2.04)	1.20 (0.72-2.00)	0.476
Highly educated	1.64 (1.23-2.17)	1.58 (1.15-2.17)	0.005	0.89 (0.56-1.42)	0.99 (0.60-1.66)	0.977
Family history of dementia	1.66 (1.19-2.31)	1.93 (1.29-2.88)	0.001	1.58 (0.83-2.61)	1.91 (1.05-3.49)	0.034

Odds ratio (95% confidence intervals). CSF = cerebrospinal fluid. *APOE* = Apolipoprotein E gene. Shown effect sizes are: Age per 5 years older at baseline, *APOE* ε4 in contrast to no *APOE* ε4, male in contrast to female, highly educated in contrast to low or normal level educated, family history for dementia positive in contrast to family history for dementia reported.

Table S3 AUC on multivariate model figure 2

	Multivariate model figure 2	
Cohort	Enrolment (AUC)	Decreased CSF Aβ +ve^ (AUC)
Toulouse Registry*	0.57 (0.47-0.67)	0.77 (0.65-0.89)
ALFA Study	0.62 (0.55-0.68)	0.66 (0.55-0.77)
Generation Scotland	0.70 (0.64-0.76)	0.88 (0.79-0.96)
pilotHO.nl	0.63 (0.54-0.71)	0.71 (0.58-0.84)

Models included: Multivariate AUCs calculated with pROC package in R of glm models (family=binominal, with DeLong confidence intervals). CSF = cerebrospinal fluid. Age at baseline, *APOE* ε4 status, gender, highly educated in contrast to low or normal level educated, status on family history for dementia. * No *APOE* genotype included in enrolment analysis.

Table S2 AUC for binominal ROC curves of table 4

	Toulouse Registry		ALFA Study		Generation Scotland		plotHO.nl	
Sample size	n=158	n=64	n=271	n=124	n=1,947	n=61	n=220	n=75
Outcome	Enrolment*	CSF Aβ +ve^	Enrolment*	CSF Aβ +ve^	Enrolment*	CSF Aβ +ve^	Enrolment*	CSF Aβ +ve^
Age	0.54 (0.45-0.64)	0.69 (0.56-0.82)	0.49 (0.42-0.56)	0.52 (0.40-0.64)	0.55 (0.49-0.61)	0.74 (0.62-0.87)	0.58 (0.50-0.65)	0.57 (0.42-0.70)
Male or Female	0.48 (0.41-0.56)	0.62 (0.51-0.73)	0.59 (0.53-0.64)	0.59 (0.49-0.68)	0.58 (0.51-0.63)	0.54 (0.41-0.67)	0.48 (0.42-0.55)	0.60 (0.47-0.70)
Education level	0.46 (0.37-0.54)	0.54 (0.41-0.66)	0.46 (0.40-0.51)	0.51 (0.41-0.61)	0.41 (0.35-0.47)	0.56 (0.42-0.68)	0.48 (0.43-0.53)	0.53 (0.42-0.62)
APOE ε4 Genotype	NA	0.68 (0.56-0.79)	0.55 (0.49-0.61)	0.57 (0.47-0.66)	0.54 (0.48-0.60)	0.72 (0.60-0.83)	0.49 (0.42-0.56)	0.64 (0.52-0.75)
Yes/No Family history for Dementia	0.50 (0.42-0.57)	0.51 (0.39-0.63)	0.50 (0.47-0.52)	0.55 (0.52-0.58)	0.40 (0.36-0.46)	0.62 (0.49-0.75)	0.56 (0.41-0.54)	0.57 (0.46-0.68)
Yes/No Subjective Cognitive Decline	0.42 (0.36-0.48)	0.58 (0.48-0.67)	0.49 (0.44-0.53)	0.51 (0.44-0.58)	NA	NA	0.49 (0.43-0.54)	0.57 (0.45-0.67)
Yes/No Low Memory Score	0.54 (0.39-0.55)	0.76 (0.56-0.81)	0.50 (0.44-0.55)	0.53 (0.44-0.62)	0.47 (0.43-0.53)	0.50 (0.39-0.62)	0.50 (0.44-0.55)	0.52 (0.44-0.60)

CSF = cerebrospinal fluid. Univariate analysis AUCs calculated with pROC-package in R of glm models (family=binominal, with DeLong confidence intervals). EPAD-LCS = EPAD longitudinal cohort study (trial-ready cohort). *APOE* = Apolipoprotein E gene. Shown effect sizes are: Age per 5 years older at baseline, *APOE* ε4 in contrast to no *APOE* ε4 genotype, male in contrast to female, highly educated in contrast to low or normal level educated, family history for dementia positive in contrast to family history for dementia reported.

4

Chapter 4

Grey matter networks, a potential endpoint for trials

Chapter 4.1

Grey matter networks decline over the disease course of autosomal dominant Alzheimer disease

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As submitted for publication

Abstract

INTRODUCTION: Structural grey matter covariance networks provide an individual quantification of morphological patterns in the brain. These networks are disrupted in sporadic Alzheimer disease, and show associations with early Alzheimer disease pathological changes and cognitive decline. Therefore, these networks might be disease progression markers. However, it remains unclear when and how grey matter networks change with disease progression. We investigated these questions in autosomal dominant Alzheimer disease mutation carriers, whose conserved age at dementia onset allows individual staging based upon their estimated years to symptom onset.

METHODS: From the Dominantly Inherited Alzheimer Network observational cohort, we selected T1-weighted MRI scans from 269 mutation carriers and 170 non-carriers (mean age 38±15 years, mean estimated years to symptom onset -9±11), of whom 237 had longitudinal scans with a mean follow-up of 3.0 years. Single-subject grey matter networks were extracted, and we calculated for each individual the network properties which describe the network topology, including the size, clustering, path length and small worldness. We determined at which time point mutation carriers and non-carriers diverged for global and regional grey matter network metrics, both cross-sectionally and for rate of change over time.

RESULTS: Based on cross-sectional data, the earliest difference was observed in path length which was decreased for mutation carriers in the precuneus area at 13 years and on a global level 12 years before estimated symptom onset. Based on longitudinal data, we found the earliest difference between groups on a global level 6 years before symptom onset, with a greater rate of decline of network size for mutation carriers. We further compared grey matter network measures with established biomarkers for AD (i.e., amyloid accumulation, cortical thickness, brain metabolism, and cognitive function). We found that greater amyloid accumulation at baseline was associated with faster decline of network measures over time, and decline in grey matter network measures over time was accompanied by decline in brain metabolism, cortical thinning, and cognitive decline.

CONCLUSION: In summary, grey matter networks deteriorate in autosomal dominant Alzheimer disease in a similar fashion as in sporadic Alzheimer disease, and the network measures show decline over time prior to estimated symptom onset. These data suggest that single-subject networks obtained from structural MRI scans form an additional non-invasive tool for understanding the substrate of cognitive decline and measuring progression from preclinical to severe clinical stages of Alzheimer disease.

1 Introduction

In order to advance clinical trials to slow or halt Alzheimer disease, the most frequent cause of dementia [1], it is important both to understand the evolution of pathophysiological changes occurring and to develop disease progression markers [2]. Current biomarkers reliably detect Alzheimer disease pathology [3], however predicting and monitoring disease progression remains difficult. Brain networks are linked to cognitive function [4-6], and may offer insights into disease progression in Alzheimer disease.

One way to measure of brain networks is by determining the similarity of grey matter morphological measures between brain regions across individuals, i.e., grey matter covariance networks [7-9] (Panel 1). This approach is based on the notion that brain regions involved in distinct cognitive functions tend to develop in a similar way, possibly due to shared neurotrophic factors [10-12]. Common developmental trajectories and functional coactivation result in similar grey matter tissue properties, as measured on structural MR imaging [13-15]. These covariance patterns are related to normal cognition [16, 17], and reveal in healthy individuals an optimal, 'small-world', organization by graph theory description [18, 19]. In sporadic Alzheimer disease dementia, grey matter networks are disrupted, showing a less optimal, random organization [20-22]. In predementia stages, such network disruptions predict clinical progression and cognitive decline [23, 24]. The presence of amyloid β (Aβ) pathology in cognitively normal individuals has also been associated with grey matter network alterations [25-27]. Together, these observations suggest that these networks change over the course of Alzheimer disease, from early stages, and that individual grey matter networks could possibly be used to monitor disease progression. However, as previous findings were based on one-time grey matter network extractions, it remains unclear whether, and when, these networks change within individuals as they progress in their disease.

A complication when studying sporadic Alzheimer disease is the difficulty of placing presymptomatic individuals on their disease timeline [28-32]. This issue is less problematic for carriers of a genetic mutation that causes autosomal dominant Alzheimer disease, because the age at onset of dementia can be estimated, from the age at onset in family members or carriers of the same specific mutation type. The estimated years to symptom onset (EYO) can serve as a proxy for disease duration [33, 34]. Using this paradigm, previous work demonstrated that Aβ aggregation starts more than two decades before dementia onset [35-37]. Closer to symptom onset, individuals show accelerated hypometabolism and cortical thinning, which is followed by cognitive decline [38-40]. When during these processes grey matter networks start to decline remains unknown.

Here, we investigated for the first time single-subject grey matter networks over the course of autosomal dominant Alzheimer disease. We assessed when, and how, the networks change as a function of EYO, both cross-sectionally and longitudinally, on a global and regional level. To understand the relationship between grey matter network changes and disease progression, we also investigated how the networks alter with established Alzheimer disease markers.

2 Materials and methods

2.1 DIAN study design and participants

In the worldwide Dominantly Inherent Alzheimer Network (DIAN) longitudinal cohort study, families with individuals carrying a PSEN1, PSEN2 or APP mutation undergo genetic testing and repeated clinical, cognitive, fluid, and brain imaging assessments. The non-carrier family members act as an inherent control group. Participants generally have study visits every three years at earlier disease stages and these assessments become yearly when either symptoms are present, or they are within three years of their EYO. DIAN protocols had supervisory approval from the ethical review board of Washington University in St. Louis, and all participants gave informed consent. For this study, we selected data from all participants who had undergone at least one MRI scan that passed quality control in the 12th data freeze. Families with the Dutch or Flemish APP mutation were excluded because these mutations result in a different phenotype, with predominantly cerebral amyloid angiopathy.

2.2 Estimated years to symptom onset (EYO)

We calculated the EYO for mutation carriers and non-carriers identically: The EYO was defined as the mutation-specific mean age at onset subtracted by the individuals' visit age [34]. In case of an unknown mutation-specific age at onset, the parental age at disease onset, reported by the participant, was used instead. For example, if the mean age at symptom onset for a specific mutation is 50 years, then a 35 year old individual would have an EYO of -15. For the carriers of the ADAD mutation, this indicates that the individual is expected to show clinical symptoms of Alzheimer disease 15 years later.

2.3 Clinical evaluation and cognition

Disease severity was measured using the Clinical Dementia Rating scale (CDR) [41], administered to the participant and study partner by blinded raters. Participants were classified as being unimpaired (global CDR score=0) or symptomatic (global CDR 0.5, 1, 2 & 3). In addition, cognitive function was summarized using a cognitive composite developed in the DIAN project [42], consisting of the average of equally weighted z-scores of the Logical Memory delayed recall total score from the Wechsler Memory Scale-Revised, DIAN Word List Test delayed free recall score, Digit Symbol Coding total score from the Wechsler Adult Intelligence Scale-Revised Digit Symbol Substitution Test, and the total score from the Mini Mental State Examination.

2.4 MR imaging acquisition and preprocessing

MRI T1-weighted scans (1.1 x 1.1 x 1.2 mm3 voxels, repetition time = 2300 ms, echo time = 2.95 ms, flip angle 9°) were acquired according to Alzheimer Disease Neuroimaging Initiative (ADNI) protocols [43]. We segmented T1 images into grey and white matter and CSF, using the Statistical Parametric Mapping software version

12 (SPM12; Wellcome Trust Centre for Neuroimaging, UCL Institute of Neurology, London, UK). All segmentations were checked visually, after which 51 scans were removed due to failed segmentations or severe motion artifacts. Native space grey matter segmentations were resampled into 2 x 2 x 2 mm3 voxels. This voxel-wise data was used as input for connectivity analyses.

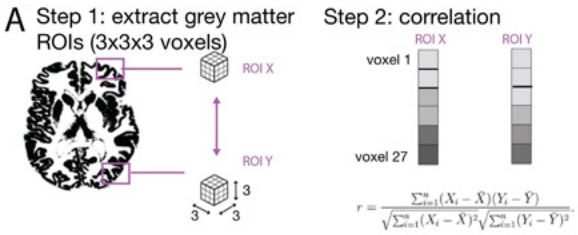
2.5 Single-subject Grey Matter Networks and Metrics

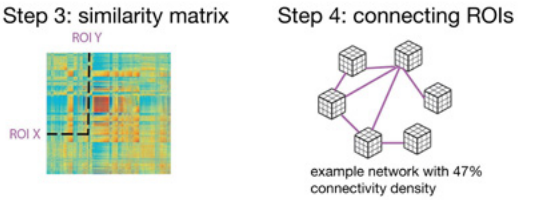
Grey matter networks were computed according to a previously published, automated pipeline [7] that includes two steps figurated in Panel A: (1) grey matter network extraction (https://github.com/bettytijms/Single_Subject_Grey_Matter_Networks; implemented in Matlab2016b (MathWorks, Natick, MA)), and (2) graph theory-based metric calculation [7, 44]. To extract single-subject grey matter networks, we parcellated each individual's native space grey matter segmentation into 6 x 6 x 6 mm3 cubes, containing 27 voxels. These non-overlapping cubes serve as the 'nodes' in the network. Connections between each pair of cubes across an individual's scan were established by calculating the Pearson's correlation coefficient between the corresponding voxels. This approach takes into account both the grey matter probability (i.e. from the tissue segmentation) as well as the spatial information present in 27 voxels within each cube. All correlations were stored in a matrix, and the presence or absence of connections between nodes was dichotomized according to an individualized threshold that ensured a maximum of 5% spurious connections for each individual [7].

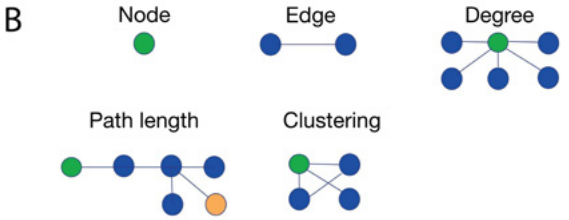
For each individual's binarized grey matter network, we calculated graph theory metrics describing the global network properties: size, degree, connectivity density, clustering coefficient, path length, normalized clustering, normalized path length, and small world coefficient (see Panel 1 for explanation of these metrics). We also calculated regional network properties. In order to aid comparability with other studies previously performed in DIAN, regional network metrics were calculated within each region of the Desikan-Killiany atlas [45]. The regional masks were obtained by first parcellating each individual's T1 image into 34 anatomical regions of interest (ROIs) from the Desikan atlas using Freesurfer 5.3 [46] (http://surfer.nmr.mgh.harvard.edu). The Freesurfer output was then aligned to the native space T1 using FSL (https://fsl.fmrib.ox.ac.uk/fsl), and this transform was used to register the parcellation into native space. The network values of the degree, clustering coefficient, and path length were subsequently averaged within a region. Graph theory metrics were calculated using scripts from the brain connectivity toolbox (https://sites.google.com/site/bctnet/),

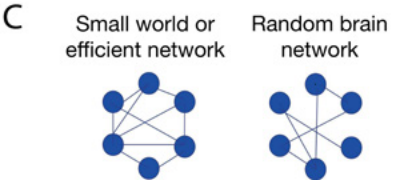
Panel 1 Grey matter network metrics

A. Grey matter network extraction from the individual brain segmentation (described in text)









B. The sum of the number of nodes, i.e., the number of cubes, is the size of the network. The degree is the average number of connections per node. The connectivity density is the percentage of the number of connections in the network compared to the maximum number of connections possible. The clustering coefficient of a node describes the proportion of connections between neighbors for every node. For example, in case a node connects to 3 other nodes, there are 3 possible connections between those 3 adjacent nodes. If only 1 connection is present between 2 of the 3 other nodes, the clustering of the primary node is 1 out of 3, 0.33. Global clustering is determined by taking averaging clustering values across all nodes. Path length is the mean of the shortest paths for a node to reach every other node in the network. The global path length is the average path length across all nodes.

C. Normalized clustering and normalized path length describe how on a global level a network organization differs from a randomly organized network. The networks are randomized by rewiring the connections randomly in each network, while keeping intact the total number of nodes and degrees [47]. The network's observed clustering and path length are divided by the clustering and path length values, respectively, of averaged random networks to obtain the normalized values. Lastly, the small world coefficient is the normalized clustering divided by the normalized path length. The network has the "small world property" if this ratio is higher than 1, indicating a path length close to the random networks, yet a greater then random clustering. This is optimal, because of fast exchange of information between remote clusters, and specialized information processing within clusters.

(Picture adapted from Verfaillie, HBM 2018, with permission)

2.6 Other DIAN imaging data

We examined regional data for Aβ using PET imaging with 11C-Pittsburgh Compound B (Aβ PET), glucose metabolism with 18F-Fluorodeoxyglucose PET (FDG-PET), and cortical thickness and volumes from structural MRI. Details on data processing have previously been described [36]. The Freesurfer ROIs were used to process the amyloid and FDG-PET data. PET data are processed using a cerebellar grey reference region and partial volume corrected using a geometric transfer matrix approach [48, 49]. In this study, we utilized the MRI precuneus cortical thickness, the precuneus Aβ PET, and to match a previously defined meta-ROI, the average of the left and right isthmus cingulate and inferior parietal region in FDG-PET for crossmodal comparison with grey matter network properties [50].

2.7 Statistical analyses

We compared mutation carriers and non-carriers to determine (1) the EYO at which grey matter network metrics showed cross-sectional differences between groups, and (2) the EYO at which the groups had a different rate of change over time by fitting linear mixed effects models. Specifically, we used Bayesian inference methods [36, 51] to determine the EYO point that 99% credible intervals of the difference distribution did not overlap 0. To allow for non-linear effects, without assuming a particular shape, we applied a restricted cubic spline with knots at the 0.10, 0.50 and 0.90 of the EYO distribution, also described previously [36], that included a linear term (EYOlinear) and a cubic term (EYOcubic). Cross-sectional models contained fixed terms for EYO, mutation status, their interaction, and a random effect for family cluster. Longitudinal models included fixed terms for baseline EYO (two terms: EYOlinear and EYOcubic), time after baseline, mutation status and, all 2- and 3-way interactions (see formulas in Sup., p.6). Additionally, the models included random intercept terms for subject and family cluster, and a random slope for subject. The covariates whole-brain grey matter volume and sex were included as fixed terms. Equivalent to previous work, when size, degree or connectivity density were found to be associated with mutation status in any of the models, they were included as additional covariate as these variables also influence more complex network metrics [21]. Regional models were additionally adjusted for regional grey matter volume. Model parameters were estimated as previously described, applying a Hamiltonian Markov chain Monte Carlo sampling of the posterior distribution, with 10,000 iterations in 8 chains, thinning retaining 1 out of every 10 iterations, and cauchy prior in the STAN package for R [52, 53].

We examined relationships between grey matter networks and established AD markers within mutation carriers. Previous research suggested grey matter networks may be disrupted in response to Aβ accumulation, precipitating cognitive decline [26]. For this reason, our models included either precuneus PET Aβ as a predictor and grey matter network metrics as outcomes, or grey matter network metrics as a predictor and cortical thickness (precuneus), brain metabolism (meta-ROI), or cognition (DIAN cognitive composite) as the respective outcomes. These predictors and outcomes

were z-scored to the whole group. We fitted three sets of linear mixed effects models that were all adjusted for baseline grey matter volume, age, and sex, and with random intercept for family cluster, in lme4 package in R [54] (see detailed formulas in Sup., p.6). If models failed to converge, the term for family cluster was removed. Models were divided into three sections. The first were baseline comparisons. The second set were longitudinal comparisons in participants with at least 2 data points, and included additional random effects for subject intercept and slope of the predictor. The final set of models were used to evaluate whether baseline data could predict change over time in the outcome. These models had fixed effects for baseline predictor, time from baseline, and its interaction, and a random subject intercept and slope of time from baseline. We focused on the grey matter network small world coefficient, as this metric is indirectly derived from all other network metrics, and can thus be considered a summary statistic (Panel 1 p.8).

2.8 Data availability

The data of the study can be freely requested online at https://dian.wustl.edu/

3 Results

In total, 439 participants from the DIAN study, with a mean±SD age of 38±11 years and a mean±SD EYO of -9±11), had MRI scans of sufficient quality to be included in the present analyses. The group consisted of 269 (61%) ADAD mutation carriers and 170 (39%) non-carrier family members (Table 1). Of this sample, 237 (54%) participants had longitudinal MRI scans, with a mean of 2.5 scans per participant and a maximum of 6 acquired over a mean±SD 3.0±1.5 years of follow-up (clinical and PET data in Sup. Table S1).

Table 1 Group characteristics

	Non-carriers (n=170)	Asymptomatic mutation carriers (n=174)	Symptomatic mutation carriers (n=95)
Baseline age, years	38 (11)	34 (9)	46 (10)
Female, n (%)	101 (59%)	100 (57%)	50 (53%)
Estimated years to onset	-11 (12)	-14 (8)	1 (7)
MMSE	29.1 (1.2)	29.1 (1.2)	22.9 (6.6)
Total MR scans, 1/2/3/4-6, n	84/61/18/7	84/59/28/3	34/30/17/14
Follow-up time MR visits, years	3.3 (1.5)	3.2 (1.5)	2.2 (1.3)

Mean (SD), unless otherwise specified. MMSE=Mini Mental State Examination. Estimated years to symptom onset is the expected age at onset of the mutation that runs in the family.

3.1. Cross-sectional divergences between mutation carriers and non-carriers

The mutation carriers diverged from non-carriers on all grey matter network metrics, except for network size and raw path length (Figure 1, Sup. Table S2). Lower network metric values for mutation carriers relative to non-carriers were observed earliest in normalized path length at EYO -12, followed by lower normalized clustering at EYO -8.7, small world coefficient at EYO -8.4, clustering coefficient at EYO -7.5, connectivity density at EYO -5.6, and degree at EYO 0. Using the same methods, but now implemented on a regional level, the earliest divergence between mutation carriers relative to non-carriers was found for path length in the precuneus at EYO -13.1, for clustering in the superior temporal gyrus at EYO -10, and for network degree in the banks of the superior temporal gyrus at EYO -7 (Figure 3, Sup. Table S3).

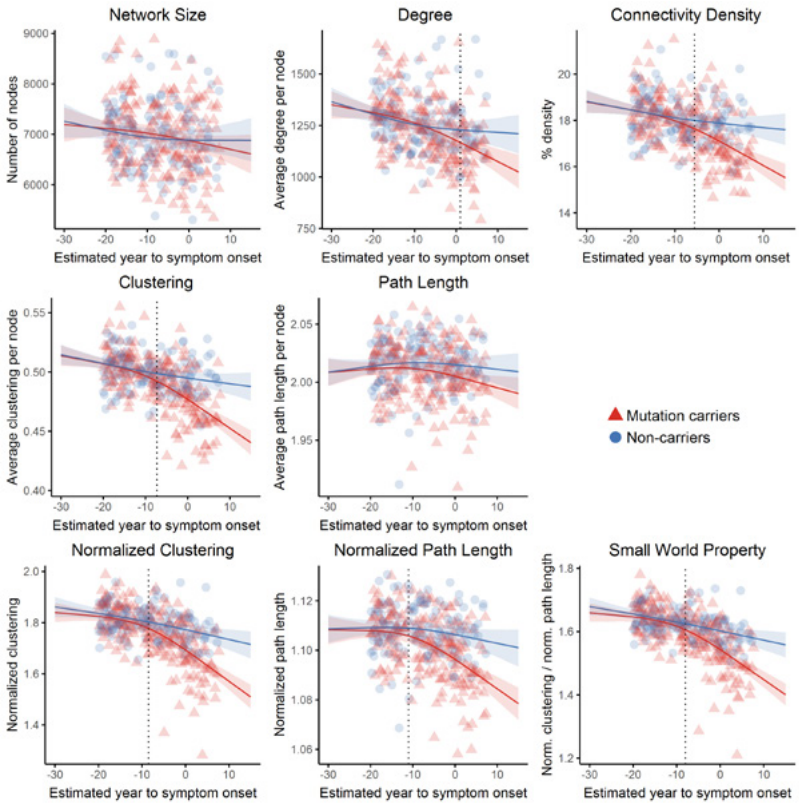


Figure 1 Grey matter networks by estimated year of onset at baseline between mutation carriers and non-carriers

The fitted lines are based on all data points extending to -38 to +20. Left of EYO 0 is before expected symptom onset, and right of EYO 0 is after expected symptom onset. The EYO were first jittered, and then the data points before -20 and after EYO +8 removed to avoid accidental unblinding of participants. Dotted line is the point of divergence between mutation carriers and non-carriers. N=439.

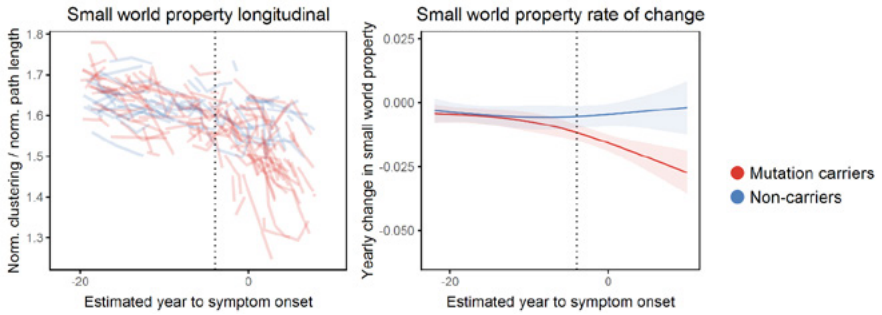


Figure 2 Rate of change grey matter network for mutation carriers and non-carriers by estimated year of onset

The fitted lines are based on all data points extending to -38 to +20. Left of EYO 0 is before expected symptom onset, and right of EYO 0 is after expected symptom onset. The EYO were first jittered, and then the data points before -20 and after EYO +8 removed to avoid accidental unblinding of participants. Dotted line is the point of divergence between mutation carriers and non-carriers.

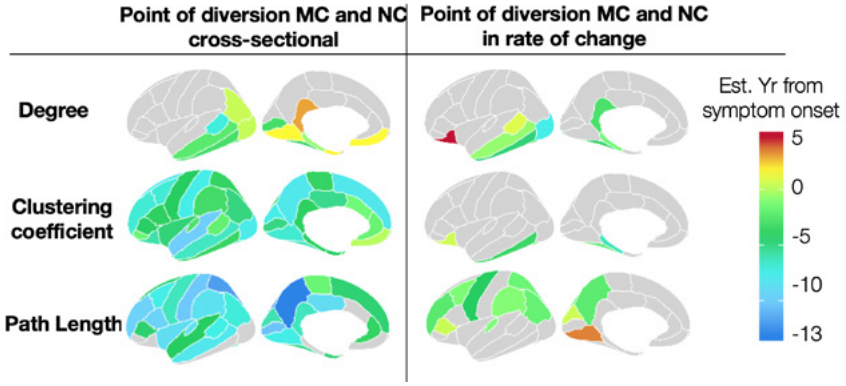


Figure 3 Regional EYO of diversion between mutation carriers and non-carriers for grey matter network degree, clustering coefficient and path length

Linear mixed models adjusted for sex, total grey matter volume and regional volume. MC=mutation carrier, NC= non-carrier. For details EYO by region see supplement table S3. N=416.

3.2 Longitudinal divergences between mutation carriers compared to non-carriers

When comparing rates of change over time, mutation carriers diverged from non-carriers by EYO for all grey matter network metrics, except connectivity density. Steeper decline for mutation carriers relative to non-carriers was detected earliest for network size, at baseline EYO -6.0, followed by small world coefficient at EYO -4.7, normalized clustering at EYO -4.6, degree at EYO -4.4, normalized path length at EYO -2.8, clustering coefficient at EYO -2.6, and path length at +1.0 (Figure 2, Sup.

Table S2 and Figure S1). When additionally adjusting for degree the estimates for network metrics yielded similar results, except for clustering coefficient, which lost significance. On a regional level, the earliest steep decline rate for mutation carriers compared to non-carriers was detected for degree in the lateral occipital gyrus at EYO -7.4, for clustering in the parahippocampal gyrus at EYO -6.2, and for path length in the precentral gyrus at EYO -4.2. (Figure 3, Supplement Table S3).

3.3 Association of grey matter networks with other neuroimaging and cognition

Established markers of Alzheimer disease showed significant relationships with the small world coefficient used as a global network summary statistic. We examined crossmodal relationships between baseline markers; over repeated measures; and whether baseline values could predict further decline in the other marker. We found that higher Aβ deposition load on PET was cross-sectionally related to a lower small world coefficient (β±SE = -0.22±0.05, p=3x10-6, Figure 4). In a longitudinal design, faster amyloid accumulation over time related to concurrent small world coefficient decline (β±SE = -0.33±0.06, p=1x10-7). Thirdly, a higher amyloid load at baseline predicted steeper decline of the small world coefficient over time (β±SE = -0.07±0.01, p=4x10-8).

Grey matter networks and the markers of Alzheimer disease progression showed significant relationships, both cross-sectionally and longitudinally (Figure 5). Specifically, a lower small world coefficient was cross-sectionally related to lower FDG-PET metabolism in the meta-ROI (B±SE = 0.44±0.08, p=2x10-7), as well as lower precuneus cortical thickness (β±SE = 0.50±0.06, p=2x10-15). For cognition, a lower small world coefficient was cross-sectionally related to lower scores on the DIAN

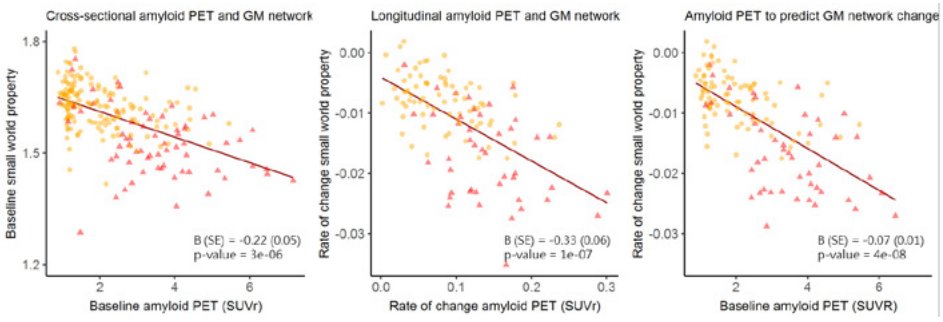


Figure 4 Association of amyloid PET with grey matter network small world coefficient in mutation carriers

For visualization purposes plotted extracted slopes with mixed model and line fitted with simple regression line in ggplot in R. Models to obtain beta and p-values specified in methods. GM network = grey matter network. Yellow circle = CDR 0 at baseline, Red triangle = CDR>0 at baseline. Amyloid PET = precuneus SUVr, Cross-sectional n= 222, Longitudinal n= 120, Predict change n= 131. For other grey matter network metrics see supplemental figure S2.

cognitive composite (β±SE = 0.28±0.08, p=3 x10-4). In a longitudinal design, decline of the small world coefficient over time related to concurrent decreases of FDG-PET metabolism (β±SE = 0.54±0.06, p=5x10-14) and faster precuneus cortical thinning (β±SE = 0.55±0.06, p=1 x10-17). A declining small world coefficient over time was related to concurrent decline on the cognitive composite (β±SE = 0.47±0.06, p=2x10-11). Thirdly, a lower small world coefficient at baseline predicted faster neurodegeneration as measured by FDG-PET metabolism (β±SE = 0.12±0.02, p=2x10-8) and precuneus cortical thinning (β±SE = 0.10±0.01, p=4x10-12), and steeper cognitive decline over time (composite B±SE = 0.08±0.02, p=2 x10-7). Associations for the other network properties can be found in Sup. Figures S2-5.

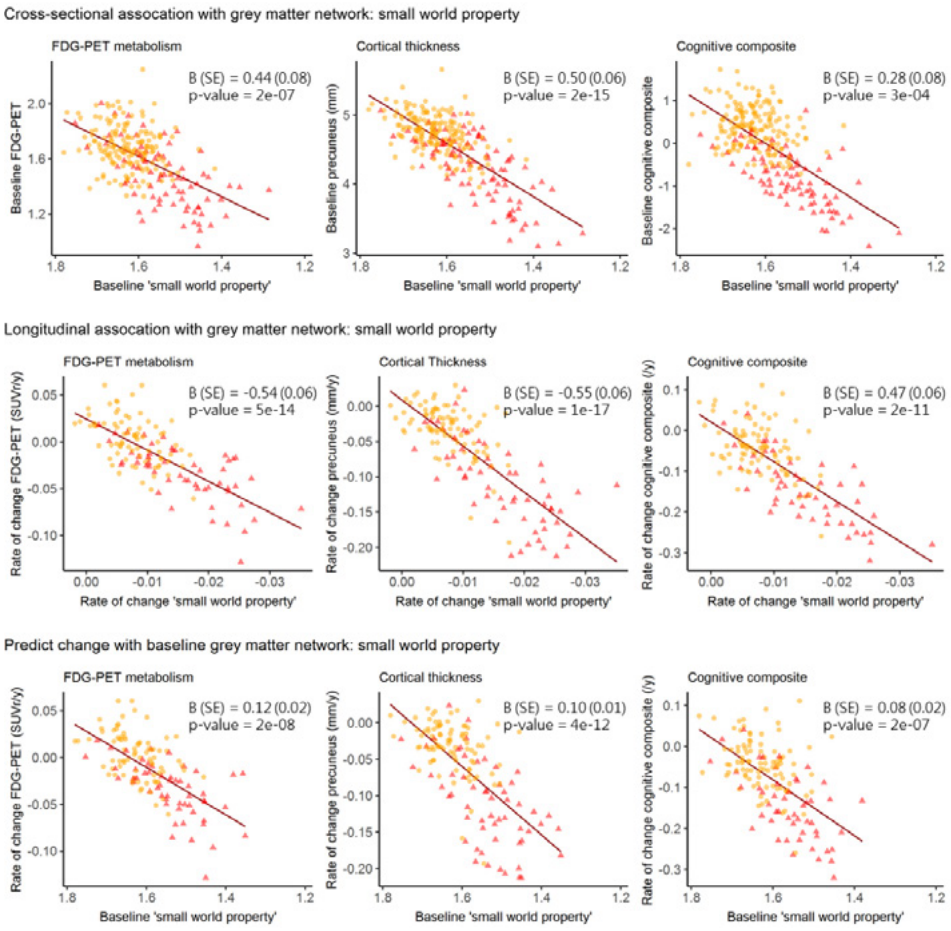


Figure 5 Associations of grey matter network small world coefficient with FDG-PET metabolism, cortical thickness and cognition

For visualization purposes plotted extracted slopes with mixed model and line fitted with simple regression line in ggplot in R. Models to obtain beta and p-values specified in methods. Inversed small world coefficient to aid visualization, see also supplemental table S4. Yellow circle = CDR 0 at baseline, Red triangle = CDR>0 at baseline. MRI thickness = cortical thickness precuneus, FDG-PET = METAROI SUVr as described in methods. DIAN composite: equally weighted z-score of Logical Memory Delayed Recall of the Wechsler memory test, DIAN Word List Test (comparable to International Shopping List Test), Digit Symbol Substitution Test and Mini Mental State Examination. Cross-sectional FDG-PET N=238 MR thickness n= 260, Cognition N=251; Longitudinal: FDG-PET n= 129 MR thickness n=146 , Cognition N= 140;Predict change: FDG-PET n= 131 MR thickness n= 146, Cognition n= 143. For other grey matter network metrics see supplemental figures S3-5.

4 Discussion

Using a single-subject approach, we found that structural grey matter networks deteriorate over the course of autosomal dominant Alzheimer disease and that moving to a more random topology closely correlates with cognitive function. When comparing mutation carriers to non-affected family members global network disruptions were detected cross-sectionally as early as 12 years before expected symptom onset. Longitudinally, increased rates of decline of network metrics were evident from 6 years before expected symptom onset. In line with our hypotheses based on cross-sectional studies in sporadic AD, grey matter network disruptions were associated with abnormalities and decline of established markers of Alzheimer disease. Thus, our grey matter network analysis in this unique cohort of autosomal dominant Alzheimer disease can contributes to our understanding of the Alzheimer disease trajectory, and indicates that our methods may potentially be a useful additional non-invasive tool for tracking disease progression. As Alzheimer disease progresses, there is substantial amyloid accumulation, volumetric loss, hypometabolism, and cognitive decline, but how grey matter networks fit into these processes remained unclear. Prior work in sporadic Alzheimer disease has shown that grey matter networks might be sensitive to biological changes during the preclinical stages of the disease [25-27]. In the current work, we observed similar alterations of grey matter networks in autosomal dominant Alzheimer disease as a function of estimated years to symptom onset. Using amyloid PET, we extended previous cross-sectional findings from studies in sporadic Alzheimer disease [26], by showing that higher baseline amyloid PET and higher amyloid accumulation rates are related to faster decline of grey matter networks over time. The consistency between sporadic and autosomal dominant Alzheimer disease strengthens the hypothesis that grey matter network disruptions are one of the downstream effects of amyloid accumulation. The networks were also related to sensitive markers of Alzheimer disease neurodegeneration and cognitive decline, in cross-sectional and longitudinal design. This suggested these processes occur, at least partly, in parallel [40], and supports the notion that grey matter network decline is a sign of progression of Alzheimer disease.

Previous studies in sporadic Alzheimer disease had suggested decline over time of grey matter networks, as there was a decrease over disease stages cross-sectionally [22, 27, 55]. Here, we show that grey matter networks decline over time

within individuals, and how decline rates start to increase with disease severity. Differences between mutation carriers and non-carriers in the rate of decline were generally detected later than cross-sectionally, which could have occurred because cross-sectional estimates across individuals by EYO may overestimate changes due to variance in the EYO measure (i.e., Some individuals at EYO -12 are actually only 5 or 6 years from actual onset) [35]. Another potential cause of cross-sectional and longitudinal estimate differences include sample sizes, with less individuals who had longitudinal data. Measurement variability over repeated measures within individuals can also have contributed to later detection of differences in the longitudinal design if these exceeded subtle rates of change. By extending follow-up time and numbers, an earlier observation decline over time may be possible.

Altering of network properties was not detected for every metric. This may be an indication that these metrics pick up different aspects of neurodegeneration. The small world measures (normalized clustering, and normalized path length and small world coefficient) showed early cross-sectional changes and seemed most sensitive to measure change over time. This is in line with network theory and previous findings in Alzheimer disease [23], which indicated that brain networks tend to become more similar to random networks over the disease course. The normalized network metrics reflect how different a network is from random, which may be why these best capture decline over time. Future studies should identify the most valid summary statistic to track longitudinal grey matter network disruptions.

On a regional level, cross-sectional network alterations were evident earliest in the parietal regions, and then spread across the brain. Most brain regions showed a difference first for path length, then for clustering and then for degree, except for the temporal regions, in which earlier and more pronounced lowering of the clustering coefficient was seen. Regional cross-sectional patterns showed early alterations for path length and clustering in areas with most pathology in autosomal dominant Alzheimer disease, including the precuneus. Regions of the default mode network also showed early alterations. Compared to previous sporadic Alzheimer disease studies, we find more widely affected connectivity, but the patterns are largely overlapping [23, 26, 56].

Compared to other structural grey matter imaging, the cross-sectional differences in the most sensitive grey matter network metrics were detected earlier than cortical thickness and volumetric measures. It was not part of this study to investigate whether grey matter networks have the same or higher sensitivity to early alterations than other structural MRI markers. Still, we adjusted for grey matter volume to assure measuring value beyond simple volumes. The rates of change were detected at a similar stage to the volumetrics, and later precuneus cortical thinning in dominantly inherited Alzheimer disease, which is the earliest region of change [36, 39]. The results merit application of grey matter networks in future deeper investigations, for example using multimodal network approaches with white matter and functional connectivity, to better understand the substrate of cognitive decline. The observation that network disruptions increase over time in a large multicenter study is relevant for clinical trials.

As the method only requires standard T1 scans and the available pipeline for network calculation, a next step is to test the approach retrospectively in clinical trial populations. One of the strengths of the current study design is the use of a previously validated method for grey matter network extraction. The unique traits of the DIAN cohort provided the ability to map changes in grey matter networks across decades of disease time. It should be noted that the estimates as a function of the expected symptom onset in dominantly inherited Alzheimer disease are influenced by sample size. Still, this method provides a way to detect and compare changes due to Alzheimer disease before symptom onset, and combine different families. Additionally, the rich characterization of DIAN participants provided the ability to relate observed changes in networks to other neuroimaging markers of pathology as well as cognition. A potential limitation is that our study included an average time period of 3 years in the longitudinal cohort, which may not be enough time to reliably measure changes due to Alzheimer disease in its very early stages. Yet, we show the longitudinal analysis of structural grey matter networks alongside of the cross-sectional results, which to the best of our knowledge has not been studied before and warrants further investigation of how grey matter networks deteriorate over time in sporadic Alzheimer disease.

In conclusion, in autosomal dominant Alzheimer disease individual grey matter networks are robustly associated with Alzheimer disease severity and progression as shown by the associations with EYO, amyloid accumulation, rate of neurodegeneration, and cognitive decline. These data suggest that single-subject grey matter networks obtained from structural MRI scans provide an additional, non-invasive tool for understanding and measuring progression from preclinical to severe clinical stages of Alzheimer disease. These grey matter networks can reflect the asynchronous start of brain pathology following Alzheimer disease-related cellular damage and inflammatory processes, informing about changes in grey matter covariance [56].

Acknowledgements

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Declarations

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Supplemental data Chapter 4.1

Table S1 Summary data other modalities

		Asymptomatic mutation carriers (N=174)	Symptomatic mutation carriers (N=95)
N observations per participant	Amyloid PET scans, 1/2/3/4-7	83/ 58/ 16/ 3	31/ 28/ 11/ 6
	FDG-PET scans, 1/2/3/4-7	88/ 54/ 21/ 3	28/ 33/ 11/ 9
	Cognitive composite, 1/2/3/4-7	76/ 61/ 27/ 5	33/ 22/ 12/ 16
Baseline value	Amyloid PET precuneus, SUVr	2.0 (1.0)	3.5 (1.4)
	FDG-PET, DIAN METAROI	1.68 (0.16)	1.46 (0.23)
	Cognitive composite, z-score	0.37 (0.50)	-0.83 (0.64)
	Cortical thickness precuneus	4.8 (0.3)	4.2 (0.5)
	Total grey matter volume*1000	627 (64)	567 (72)

Mean (SD), unless otherwise specified. DIAN METAROI = Mean of 4 Desikan regions of Freesurfer: isthmus cingulate and inferior parietal, both left and right hemisphere. DIAN composite: equally weighted z-score of Logical Memory Delayed Recall of the Wechsler memory test, DIAN Word List Test (comparable to International Shopping List Test), Digit Symbol Coding and Mini Mental State Examination.

Table S2 Divergence between carriers and non-carriers by estimated years to symptom onset (EYO) cross-sectional and longitudinal, with sensitivity analysis for different models

		EYO of divergence			
	Covariates: sex 0 = male - gm volume mean-centered	Covariates: - sex 0 = female - gm volume mean-centered	Covariates: - sex 0 = male - gm volume mean-centered - average degree mean-centered	No covariates. with family term	No covariates. no family term
	Cross-sectional				
Size	no diff	no diff	n/a	no diff	
Average degree	0.0	+1.0	n/a	-1.2	
Connectivity Density	-5.6	-5.6	n/a	-5.8	
Average clustering	-7.5	-7.3	-8.6	-7.6	
Normalized clustering	-8.7	-8.5	-9.6	-7.7	
Average path length	no diff	no diff	n/a	-3.5	
Normalized path length	-12	-11.9	-12.4	-8.8	
Small world property	-8.4	-8	-7.7	-7.5	
	Longitudinal				
Size	-6.0	-6.3	n/a	-6.1	-6.0
Average degree	-4.4	-4.0	n/a	-3.4	-3.5
Connectivity Density	no diff	no diff	n/a	no diff	no diff
Average clustering	-2.6	-3.3	no diff	-3.5	-2.8
Normalized clustering	-4.6	-4.4	-4.5	-4.7	-4.7
Average path length	+1.0	+0.9	-4.8	+1.1	+1.2
Normalized path length	-2.8	-3.0	-4.2	-2.4	-2.4
Small world property	-4.7	-4.7	-4.4	-4.3	-4.4

Output cross-sectional analysis 99% credible intervals of the difference line; gm volume = total grey matter volume; gm volume and average degree were mean-centered; all models include a random family intercept and in the longitudinal models also for subject intercept and slope.

Table S3 Regional point of divergence between carriers and non-carriers by estimated years to symptom onset (EYO) cross-sectionally and longitudinal rate of change

Lobe	Region	Cross-sectionally			Longitudinal rate of change		
		Degree	Clustering	Path length	Degree	Clustering	Path length
F/P/T	Insula	-	-4.7	-8.7	-	-	-
F	Caudal middle frontal	-	-6.8	-7.4	-	-	-1.1
F	Frontal pole	-	-0.8	-	-	-	-
F	Lateral orbitofrontal	-	-5.8	-	+4.7	+0.5	-
F	Medial orbito frontal	+1.7	+0.0	-	-	-	-
F	Para central	-	-3.8	-1.7	-	-	-
F	Pars opercularis	-	-4.0	-9.5	-	-	-
F	Pars orbitalis	-	-1.7	-	-	-	-
F	Pars triangularis	-	-4.3	-4.5	-	-	+0.4
F	Pre central	-	-4.2	-6.3	-	-	-4.2
F	Rostral middle frontal	-	-6.8	-9.7	-	-	-2.4
F	Superior frontal	-	-7.9	-4.6	-	-	-
F (C)	Caudal anterior cingulate	-	-1.4	-	-	-	-
F (C)	Rostral anterior cingulate	-	-1.8	-3.8	-	-	-
P	Inferior parietal	+0.4	-4.9	-6.9	-	-	-1.9
P	Post central	-	-7.4	-10.3	-	-	-
P	Precuneus	-	-7.9	-13.1	-	-	-2.3
P	Superior parietal	-	-3.8	-12.0	-	-	-1.7
P	Supramarginal	-	-3.3	-8.4	-	-	-1.0
P (C)	Isthmus cingulate	+3.0	-4.0	-6.3	-3.0	-	-
P (C)	Posterior cingulate	-	-5.2	-8.8	-	-	-
O	Cuneus	-	-7.4	-6	-	-	+0.5
O	Lateral occipital	+0.3	-7.6	-	-7.4	-	-
O	Lingual	+1.7	-6.7	-7.3	-	-	+3.5
O	Pericalcarine	-3.2	-5.0	-10.5	-	-	-
T	Banks superior temporal	-7.0	-2.0	-7.2	0.9	-	-
T	Entorhinal	+1.8	-4.1	-4.7	-	-	-
T	Fusiform	-3.5	-6.1	-5.3	-4	-2.1	-
T	Inferior temporal	-3.1	-3.7	-	-4.7	-3.6	-
T	Middle temporal	-2.4	-6.4	-7.9	-0.7	-	-
T	Parahippocampal	-0.7	-4.0	-	-1.4	-6.2	-
T	Superior temporal	-	-10.0	-4.2	-	-	-
T	Temporal pole	-	-4.2	-	-	-	-
T	Transverse temporal	-	-2.9	-8.4	-	-	-1.1

Output cross-sectional analysis 99% credible intervals of the difference line; all models include a random family intercept and in the longitudinal models also for subject intercept and slope. The models for degree are adjusted for baseline grey matter volume, mean-centered, baseline regional volume, mean-centered, and sex (0=male), and for clustering and path length also for baseline degree, mean-centered. T=temporal lobe, P=parietal lobe, F=frontal lobe, C=cingulate, O=occipital. EYO = estimated years to symptom onset.

Table S4 Associations between grey matter network small world property and other imaging and clinical markers in mutation carriers

Model	Outcome	Predictor	Beta (SE)	p-value
Model 1 Cross-sectional	Small world property	Amyloid PET	-0.22 (0.05)	3e-06
	FDG-PET	Small world property	0.44 (0.08)	2e-07
	MR thickness	Small world property	0.50 (0.06)	2e-15
	Cognitive composite	Small world property	0.28 (0.08)	3e-04
Model 2 Longitudinal; fixed effect predictor	Small world property	Amyloid PET	-0.33 (0.06)	1e-07
	FDG-PET	Small world property	0.54 (0.06)	5e-14
	MR thickness	Small world property	0.55 (0.06)	1e-17
	Cognitive composite	Small world property	0.47 (0.06)	2e-11
Model 3 Predict change with baseline value; fixed effect interaction predictor over time after baseline	Small world property	Amyloid PET	-0.07 (0.01)	4e-08
	FDG-PET	Small world property	0.12 (0.02)	2e-08
	MR thickness	Small world property	0.10 (0.01)	4e-12
	Cognitive composite	Small world property	0.08 (0.02)	2e-07

Linear models adjusted for baseline age, sex, total grey matter volume, and if possible family cluster. Additionally, in model 2 random effect for subject intercept and subject predictor slope, and in model 3 subject intercept and slope after baseline, plus fixed effect time after baseline and interaction between time and predictor. Amyloid PET = precuneus SUVr, MR thickness = cortical thickness precuneus, FDG-PET = METAROI SUVr as described in methods. DIAN composite: equally weighted z-score of Logical Memory Delayed Recall of the Wechsler memory test, DIAN Word List Test (comparable to International Shopping List Test), Digit Symbol Coding and Mini Mental State Examination. All predictor and outcome variables were scaled.

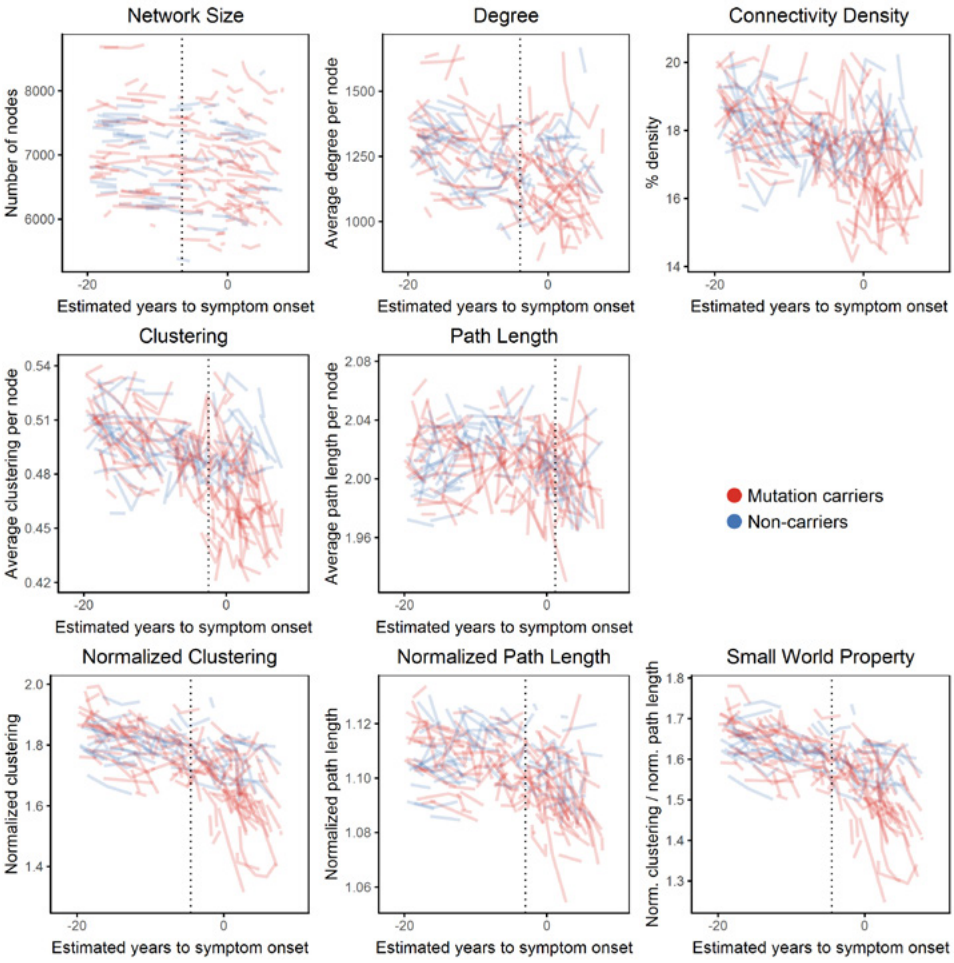

Figure S1 Raw data longitudinal grey matter networks with estimated points of divergence between mutation carriers and noncarriers

The fitted lines are based on all data points extending to -38 to +20. The graph was adapted to avoid accidental unblinding of participants, including 1 outlier removed. Left of EYO 0 is before expected symptom onset, and right of EYO 0 is after expected symptom onset. The EYO were first jittered, and then the data points before -20 and after EYO +8 removed. Dotted line is approximately the point of divergence between mutation carriers and non-carriers.

Parameters in statistical models

1 EYO comparisons:

a. Cross-sectional

$$\begin{aligned}\text{Network}_{\text{metric}} &= \beta0 + \beta1*\text{EYO}_{\text{linear}} + \beta2*\text{EYO}_{\text{cubic}} + \beta3*\text{Mutation}_{\text{status}} + \beta4 \\ &* \text{EYO}_{\text{linear}}*\text{Mutation}_{\text{status}} + \beta5*\text{EYO}_{\text{cubic}}*\text{Mutation}_{\text{status}} \\ &+ (\text{Random intercept}|\text{Family}_{\text{cluster}}) + \beta x*\text{covariates}\end{aligned}$$

b. Longitudinal

$$\begin{aligned}\text{Network}_{\text{metric}} &= \beta0 + \beta1*\text{EYO}_{\text{linear}} + \beta2*\text{EYO}_{\text{cubic}} + \beta3*\text{ time} + \beta4\text{Mutation}_{\text{status}} + \beta5 \\ &*\text{EYO}_{\text{linear}}*\text{time} + \beta6*\text{EYO}_{\text{cubic}}*\text{time} + \beta7*\text{Mutation}_{\text{status}}* \text{ time} + \beta8 \\ &*\text{EYO}_{\text{linear}}*\text{Mutation}_{\text{status}}* \text{ time} + \beta9*\text{EYO}_{\text{cubic}}*\text{Mutation}_{\text{status}}*\text{time} \\ &+ (\text{Random intercept+time }|\text{Individual}) + (\text{Random intercept}|\text{Family}_{\text{cluster}}) \\ &+ \beta x*\text{covariates}\end{aligned}$$

2 Crossmodal comparisons mutation carriers only

a. Cross-sectional

$$\begin{aligned}\text{Outcome}_{\text{\_baseline}} &= \beta0 + \beta1*\text{Predictor\_baseline} \\ &+ (\text{Random intercept}|\text{Family}_{\text{cluster}}) + \beta x *\text{covariates}\end{aligned}$$

b. Longitudinal

$$\begin{aligned}\text{Outcome} &= \beta0 + \beta1*\text{Predictor} + (\text{Random intercept+predictor}|\text{Individual}) \\ &+ (\text{Random intercept}|\text{Family\_cluster}) + \beta x*\text{covariates}\end{aligned}$$

c. Predict rate of change over time

$$\begin{aligned}\text{Outcome} &= \beta0 + \beta1*\text{Predictor}_{\text{baseline}} + \beta2*\text{time} + \beta3*\text{predictor}_{\text{baseline}}*\text{time} \\ &+ (\text{Random intercept+time}|\text{Individual}) + (\text{Random intercept}|\text{Family}_{\text{cluster}}) \\ &+ \beta x*\text{covariates}\end{aligned}$$

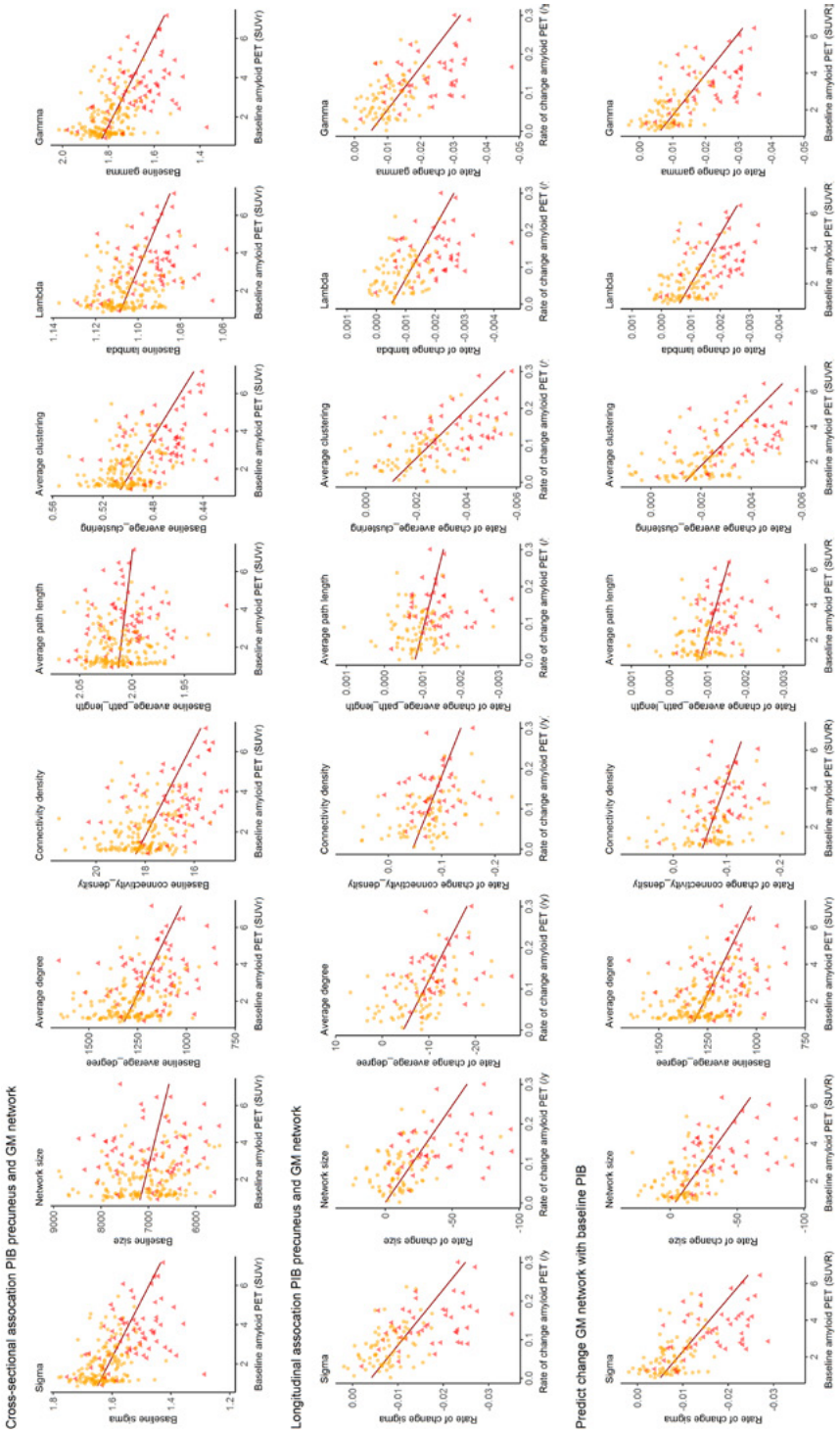

Figure S2 Comparisons PIB and other grey matter network with mutation carriers
Yellow circle = asymptomatic; red triangle = symptomatic at baseline

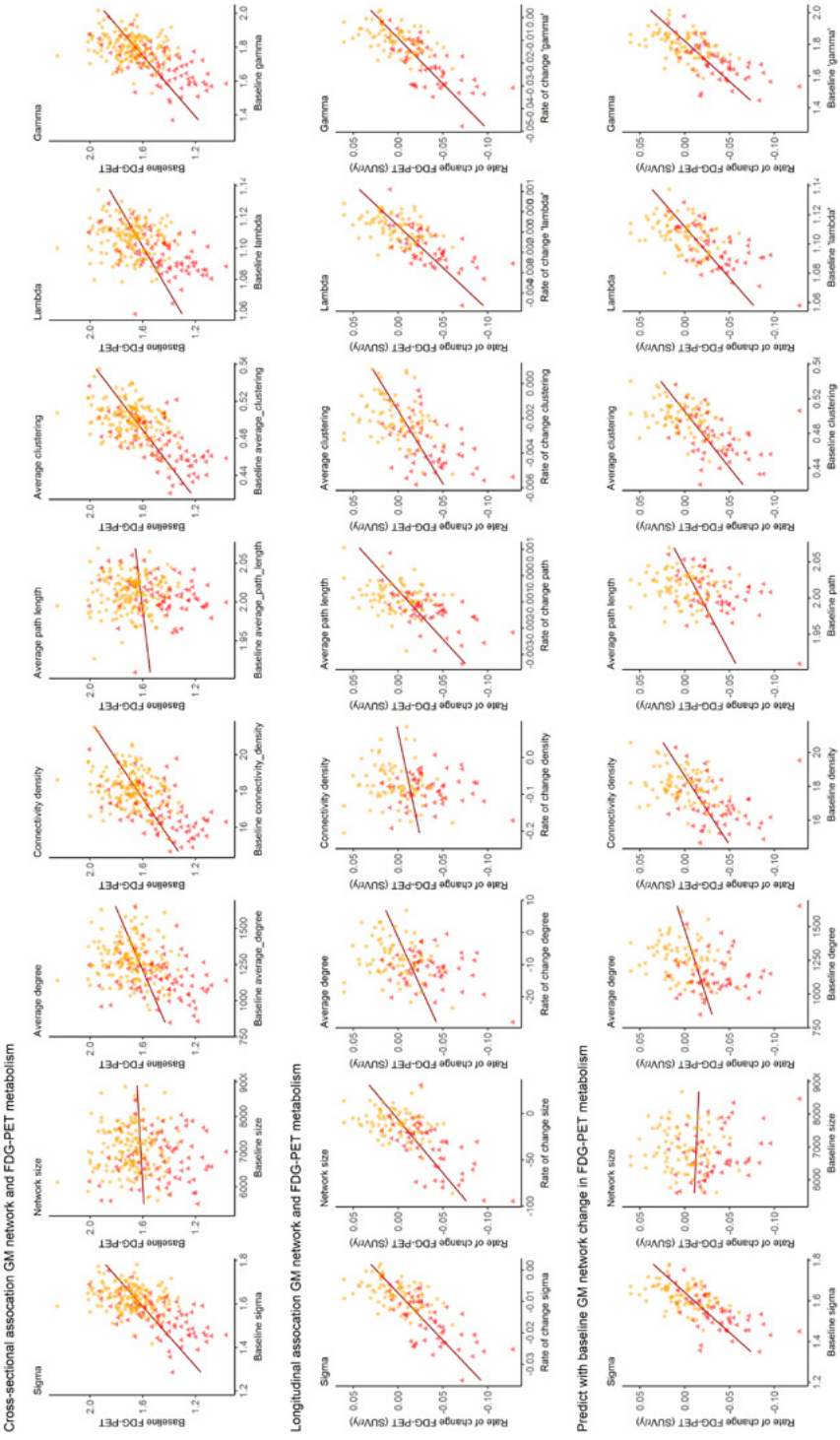

Figure S3 Comparisons other grey matter network measures and FDG metabolism in mutation carriers

Yellow circle = asymptomatic; red triangle = symptomatic at baseline

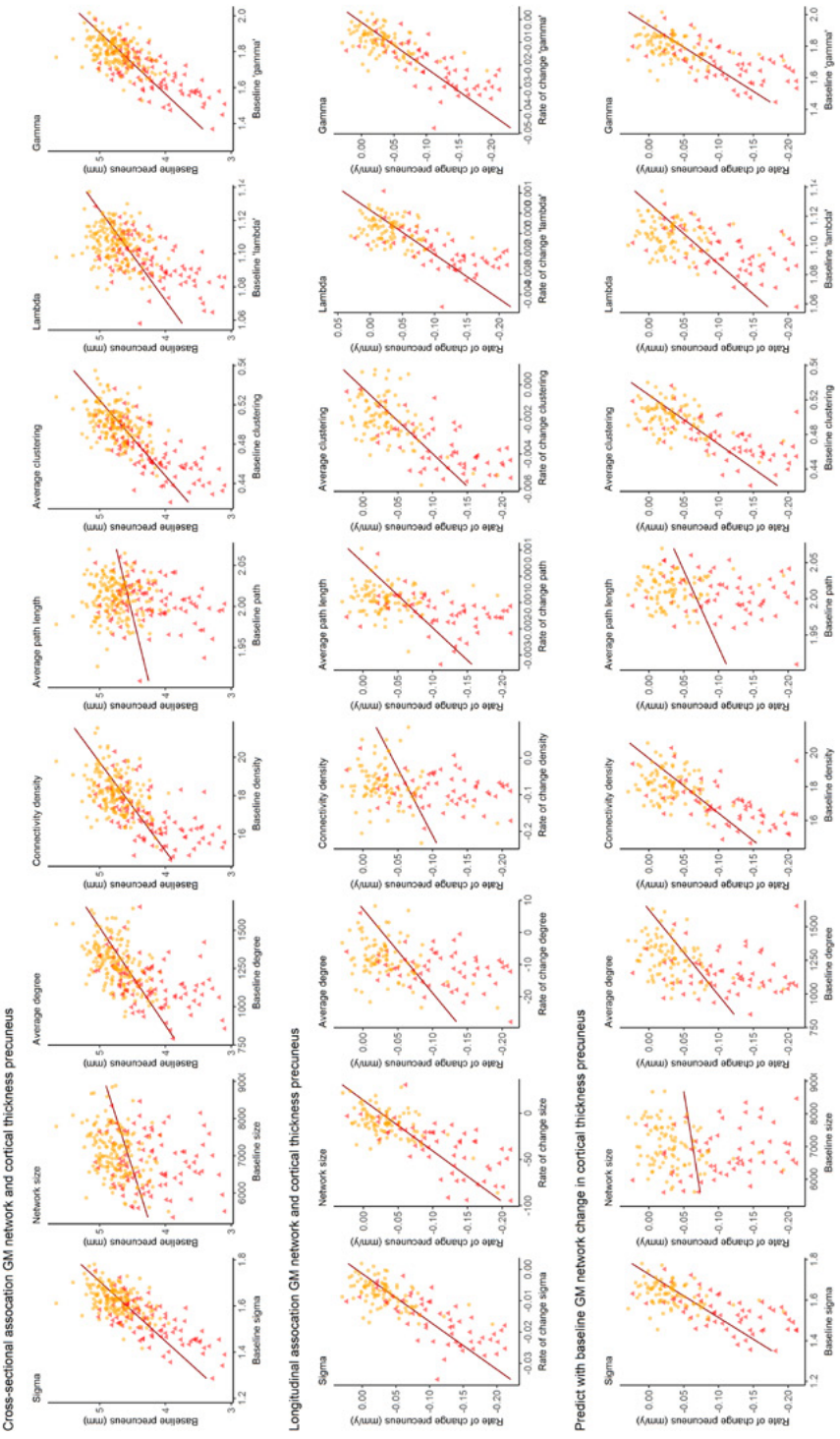

Figure S4 Comparisons other grey matter network measures and cortical thickness in mutation carriers

Yellow circle = asymptomatic; red triangle = symptomatic at baseline

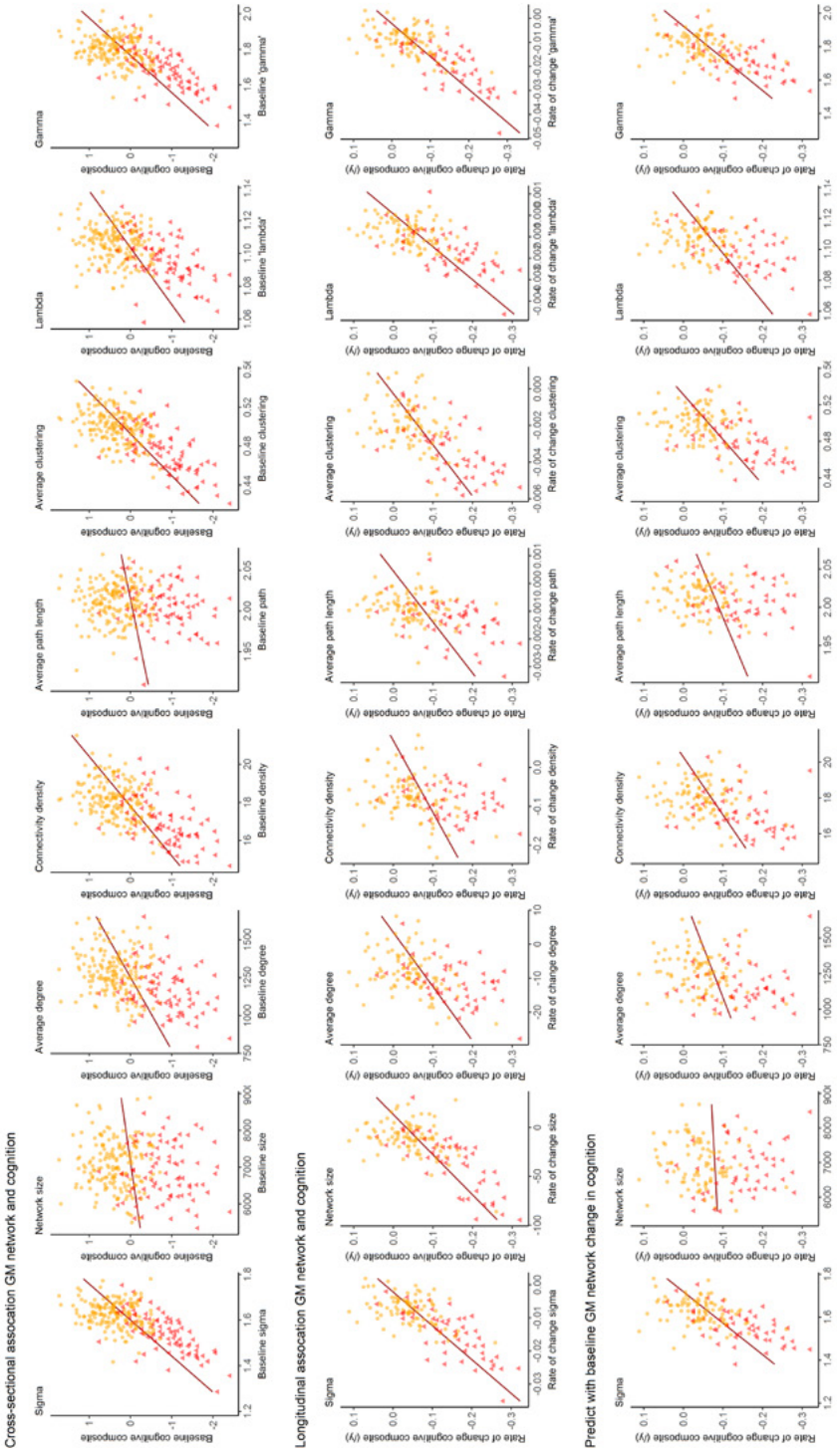

Figure S5 Comparisons other grey matter network measures and cognition in mutation carriers
Yellow circle = asymptomatic; red triangle = symptomatic at baseline

Chapter 4.2

Biological correlates of grey matter network disruption in autosomal dominant Alzheimer disease

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Abstract

BACKGROUND: Structural grey matter covariance networks are disrupted in neurodegenerative disorders such as Alzheimer disease (AD). These disruptions are related to early amyloid aggregation and cognitive decline, but the precise biological underpinnings of network changes remain unknown. Besides amyloid aggregation, many other pathological processes occur in AD, including synaptic dysfunction and loss, axonal degeneration, neuronal damage, and inflammatory processes, that may contribute to grey matter network disruptions. Therefore, we investigated how cerebrospinal fluid (CSF) proteins concentrations, reflecting these pathological processes, are associated with grey matter network disruptions in autosomal dominantly inherited AD (ADAD) mutation carriers.

METHODS: From the Dominantly Inherited Alzheimer Network (DIAN) Observational study, we included 219 mutation carriers and 136 noncarriers with both T1-weighted MRI and CSF collection. CSF biomarkers included: $A\beta_{40/42}$ ratio (amyloid aggregation), pTau (hyperphosphorylation), tTau and VILIP-1 (neuronal injury and death), SNAP-25 and neurogranin, (synaptic damage), NfL (axonal injury), YKL-40 and soluble TREM2 (neuro-inflammation). We examined relationships between CSF levels of these markers and grey matter network integrity as quantified by the small world coefficient. This measure indicates whether networks deviate from a randomly organized network. We further tested whether relationships were dependent on disease stage, and fitted the trajectory of the disease course for each of the markers.

RESULTS: Increased pTau, tTau, SNAP-25, Ng, VILIP-1, NfL and YKL-40 were associated with lower small world values in mutation carriers. NfL showed the strongest relationship with the small world coefficient (β±SE = -0.72±0.05; p<0.001). Within carriers, these relationships were not significantly different across disease stages. Abnormalities in the traditional CSF biomarkers and synaptic and neuronal injury markers preceded grey matter network disruptions by several years, while YKL-40 and NfL abnormalities co-occurred.
CONCLUSION: Our results suggest that axonal loss may contribute to disrupted grey matter networks as observed in AD.

1 Introduction

Brain areas implicated in similar functions show covariation in cortical morphology on magnetic resonance imaging (MRI), and these covariation patterns can be precisely quantified with a network approach [1-3]. In neurodegenerative diseases, such as Alzheimer disease (AD), grey matter networks become disrupted [3-5]. With increasing disease severity in AD, grey matter networks become more randomly organized, as consistently indicated by a lower small world coefficient [6]. These network disruptions are related to impaired cognition and future cognitive decline, both in sporadic and autosomal dominant AD (ADAD) [6-13]. Network disruptions can already be detected in cognitively normal individuals with amyloid aggregation (presumed preclinical AD) [6, 14, 15]. Still, the biological mechanisms that explain the deterioration of network organization remain unclear. Changes in grey matter networks could result from multiple pathophysiological processes such as synaptic dysfunction and loss, axonal degeneration, neuronal loss, and local swelling in response to infiltration of inflammatory cells. A better understanding of network disruptions over the course of AD may inform new hypotheses regarding how brain connectivity could be maintained in order to preserve cognitive function.

In cerebrospinal fluid (CSF), proteins can be measured that reflect ongoing biological processes in the brain. CSF biomarkers are used for the biological definition of AD based on abnormal concentrations of β-amyloid 1-42 (ratio of β-amyloid 1-42/1-40 [$A\beta_{42/40}$]), hyperphosphorylation of tau (181-phosphorylated fraction [pTau]), and neuronal injury (total tau [tTau]) [16]. In addition to these core AD measures, other biomarkers have robustly been related to AD, and provide information on additional pathological brain alterations occurring in the disease [17]. Increased levels of synaptosomal-associated protein-25 (SNAP-25) and neurogranin (Ng) levels are markers of pre-synaptic and post-synaptic dysfunction, respectively; visinin-like protein 1 (VILIP-1) of neuronal death; and neurofilament light chain (NfL) of axonal damage [18-23]. In addition, chitinase-3-like protein 1 (YKL-40), an astrocyte marker, and soluble TREM2 (sTREM2) [19, 20, 23, 24], a marker of activated microglia, are also elevated in AD and provide insight into inflammatory processes. It is conceivable that abnormal of levels of these markers may impact brain connectivity, but this remains largely unknown.

Here, we studied this question in carriers of ADAD genetic mutations, which has the advantage of a relatively conserved dementia onset age and few age-related co-pathologies due to the relatively young age of symptoms [25]. We assessed the associations between both the core and emerging CSF biomarkers for AD and the individual grey matter network summary statistic the 'small world coefficient'. We further tested within mutation carriers whether the relationships observed were depended on disease stage, as determined by a combination of the pTau/$A\beta_{42}$ ratio (normal, abnormal) and the clinical dementia rating score (CDR).

2 Methods

2.1 Participants and design

Data was obtained from the Dominant Inherited Alzheimer Network Observational Study (DIAN-Obs) [26]. For the DIAN study, ADAD mutation carriers (MC) (presenilin 1 [PSEN1], presenilin 2 [PSEN2] and amyloid precursor protein [APP]) and their noncarrier (NC) family members undergo longitudinal clinical and cognitive examinations, neuroimaging and biospecimen donations. We evaluated data that passed quality control and was included in data freeze 12. Families with Flemish and Dutch mutations were excluded from analyses, because these mutation result in a different phenotype, with primarily cerebral amyloid angiopathy. The study was approved by the ethical review board at Washington University, St. Louis, Missouri, USA and local IRBs. The estimated years to symptom onset (EYO) for each individual was defined as the mutation-specific (e.g. for the PSEN1 G206A mutation, the mean age at onset is 53) mean age at onset subtracted from the individuals' visit age [25]. In case mutation age of onset was unknown, the family-specific parental age of disease onset was used instead. For example, if the mean age at symptom onset is 53 years for a specific family mutation, then a 43 year old individual, regardless of mutation status, would have an EYO of -10. This indicates an individual with the mutation is expected to show clinical symptoms of AD 10 years later, and allows comparison of biomarkers with the NCs on the same timeline, as well as between MCs and NCs from different families and mutations. For the for the biomarker comparisons, we selected the first visit at which individuals had both CSF and MRI data available.

2.2 Group definitions

Participants were stratified in two ways. The first set of analyses focus on comparing all MCs to their familial NC controls. The second set of analyses staged MCs into 4 groups based upon their biomarker status and CDR [27]. Group 1 had a normal CSF ratio of pTau/$A\beta_{42}$ (< 0.019 [28]) (indicating absence of underlying brain amyloid). Group 2-4 had abnormal ratios (indicating presence of amyloid) and increasing CDRs of: group 2: CDR= 0, no impairment; group 3: CDR = 0.5, very mild dementia; group 4: CDR >= 1 mild to severe dementia.

2.3 MR preprocessing

MR scans were collected and preprocessed according the protocols of the Alzheimer's Disease neuroimaging Initiative (1.1 by 1.1 by 1.2 mm^3 voxels, repetition time = 2300, echo time = 2.95, flip angle 9°), described in detail in [29, 30]. For the network extractions, T1-weighted scans were first segmented into grey matter, white matter and CSF in native space with Statistical Parametric Mapping 12 (SPM12; Wellcome Trust Centre for Neuroimaging, UCL Institute of Neurology, London, UK). The segmentations were checked and resliced into 2mm by 2mm by 2mm voxels, and this was the input for the grey matter network extraction.

2.4 Calculation of grey matter network metrics

Single-subject grey matter network metrics were extracted from preprocessed grey matter segmentations according to previously published procedures, (https://github.com/bettytijms/Single_Subject_Grey_Matter_Networks)[2] as follows: Grey matter segmentations were parcellated into cubes of 3 by 3 by 3 voxels, and these cubes formed the nodes of the network. The Pearson's correlation coefficient was then calculated for grey matter intensities across the voxels for each pair of cubes. Next, correlation values were binarized and only significant connections retained. Finally, we calculated for each network the small world coefficient using scripts from the brain connectivity toolbox (https://sites.google.com/site/bctnet/ [31] modified for large sized networks. In this study, we use the small world coefficient, which is a whole brain summary statistic and normalized for individual differences in degree and size of networks. A network with a small world coefficient of 1 has a random organization, while a value higher than 1 indicates the networks exhibits the 'small world property'. Technically, networks are 'small world' when the level of clustering is high, while the path length to every other node is still relatively short [32, 33].

2.5 Cerebrospinal fluid markers

Participants underwent lumbar puncture after overnight fasting. Samples were collected via gravity drip in polypropylene tubes and sent on dry ice to the DIAN biomarker laboratory at Washington University. The samples were aliquoted in 0.5mL polypropylene tubes, stored at -84°C before measurements of SNAP-25, Ng, VILIP-1 and YKL-40. Additional aliquots of each sample were shipped on dry ice for the measurements of $A\beta_{40}$, $A\beta_{42}$, pTau and tTau by the Shaw laboratory at the University of Pennsylvania [34], of NfL by the Kuhle laboratory in Basel [35], and of sTREM2 by the Haass laboratory in Münich [36]. For details on the protocols, see [21, 22, 24, 37]. Briefly, $A\beta_{40}$, $A\beta_{42}$, pTau and tTau levels were determined using the automated Elecsys assay, and values of $A\beta_{40}$ and $A\beta_{42}$ outside the measurement ranges were extrapolated on the calibration curve [37]. SNAP-25, Ng and VILIP-1 were measured with antibodies developed in the laboratory of Dr. Jack Ladenson at Washington University in St. Louis, as part of micro-particle-based immunoassays using the Singulex (now part of EMD Millipore; Alameda, CA) Erenna system [22]. YKL-40 was measured with

Table 1 Demographics and baseline summary data on predictors and outcomes.

	Noncarriers (NCs)		Mutation carriers (MCs)				
	All (n=136)	NCs < 40 years old (n=81)	All (n=216)	MCs: ratio neg (n=84)	MCs: CDR 0, ratio pos (n=63)	MCs: CDR 0.5, ratio pos (n=43)	MCs: CDR 1-3, ratio pos (n=26)
Demographics							
N (%) Male	53 (39%)	32 (40%)	96 (44%)	35 (42%)	28 (44%)	19 (44%)	14 (54%)
Age, years	38 ± 12	31 ± 6	39 ± 10	32 ± 8	38 ± 9	47 ± 9	47 ± 9
EYO, years	-10 ± 12	-17 ± 9	-9 ± 11	-17 ± 8	-8 ± 7	1 ± 6	4 ± 4
Years of education, median±IQR	15 ± 3	15 ± 2	14 ± 4	15 ± 3	15 ± 4	14 ± 4	12 ± 2
CDR (0/0.5-1/2-3), N	131/5/0	0/2/0	142/66/8	79/5/0	63/0/0	0/43/0	0/18/8
MMSE, median±IQR	30 ± 1	30 ± 1	29 ± 3	29 ± 1	29 ± 2	26 ± 4	16 ± 10
Grey matter network							
Small world coefficient	1.62 ± 0.05	1.65 ± 0.05	1.59 ± 0.08	1.64 ± 0.06	1.60 ± 0.05	1.55 ± 0.08	1.46 ± 0.07
Traditional CSF markers							
$A\beta_{42}$ pg/ml	1,407 ± 466	1,292 ± 442	974 ± 634	1,526 ± 655	716 ± 279	553 ± 208	510 ± 217
$A\beta_{40}$ pg/ml	15,698 ± 4418	14,398 ± 4,204	14,862 ± 4760	15,607 ± 5,080	15,004 ± 4,749	14,483 ± 4,114	12,741 ± 4,241
pTau pg/ml	14 ± 5	13 ± 4	31 ± 23	14 ± 4	32 ± 18	46 ± 23	57 ± 28
tTau pg/ml	169 ± 55	154 ± 49	290 ± 162	177 ± 48	305 ± 120	375 ± 142	475 ± 241
Ratio $a\beta_{42/40}$	0.089 ± 0.01	0.089 ± 0.007	0.066 ± 0.035	0.099 ± 0.031	0.049 ± 0.017	0.039 ± 0.012	0.042 ± 0.015
ratio $A\beta_{42/40}$ ↓0.075, N (%)	6 (4%)	1 (1%)	144 (67%)	19 (23%)	58 (92%)	42 (98%)	25 (96%)
ratio pTau/ $A\beta_{42}$	0.010 ± 0.004	0.010 ± 0.002	0.052 ± 0.053	0.010 ± 0.004	0.051 ± 0.034	0.091 ± 0.049	0.120 ± 0.065
ratio pTau/$A\beta_{42}$ ↑0.0198, N (%)	2 (1%)	0 (0%)	132 (61%)	-	-	-	-
Emerging CSF markers							
SNAP-25 pg/ml	3.6 ± 1.3	3.2 ± 1.1	4.6 ± 1.9	3.6 ± 1.1	4.5 ± 1.5	5.2 ± 1.7	6.4 ± 2.7
Ng pg/ml	1,563 ± 741	1,447 ± 765	2,297 ± 1,212	1,638 ± 682	2,526 ± 1,109	2,673 ± 1,164	3,120 ± 1,748
NfL pg/ml	793 ± 544	564 ± 396	1,939 ± 1,762	531 ± 190	1,033 ± 650	2,630 ± 1,643	3,873 ± 1,657
VILIP-1 pg/ml	133 ± 50	122 ± 48	174 ± 79	135 ± 47	179 ± 71	198 ± 75	236 ± 114
YKL-40 ng/ml	133 ± 66	98 ± 37	173 ± 88	109 ± 37	169 ± 69	229 ± 81	280 ± 89
sTREM2, relative to reference sample	0.47 ± 0.22	0.43 ± 0.21	0.58 ± 0.29	0.42 ± 0.15	0.48 ± 0.28	0.73 ± 0.3	0.74 ± 0.3

Legend: CSF biomarkers not available for the whole sample: SNAP (n=330), Ng (n=331), VILIP1 (n=330), YKL40 (n=331), NfL (n=169 (incl. 19 with no MRI data), sTREM2 (n=164).

plate-based enzyme-linked immunoassay (MicroVue ELISA; Quidel, San Diego, CA) [22]. NfL was measured with a single-molecule array assay using the capture monoclonal antibody 47:3 and biotinylated detection antibody 2:1 (UmanDiagnostics AB, Sweden) [21]. sTREM2 was measured using the MSD platform with an in-house developed ELISA based on commercially available antibodies [24]. The sTREM2 concentrations are reported relative to the pooled sample that was loaded onto all plate, as a way to account for plate variation.

2.6 Statistical analysis

In all linear models pTau, tTau, SNAP-25, Ng, VILIP-1, YKL-40, NfL and sTREM2 were log-transformed to approach normality. To aid comparability of slope estimates, the variables were Z-transformed according to the total group. We tested the associations between the CSF biomarkers as predictors and the small world coefficient as the outcome with three linear regression models. Model 1 was adjusted for sex; Model 2 also included a fixed term for mutation status and its interaction with the predictor; Model 3 had additional adjustment for age effects. We further performed a subgroup analysis within MCs only to investigate disease stage effects, by running models that included the CSF predictor, a fixed term for the severity groups and its interaction with the predictor. We ran post-hoc pairwise comparisons using the Tukey HSD procedure. Lastly, we estimated trajectories for all markers studied by EYO, using a previously developed Bayesian inference linear mixed effect model [29, 38] to obtain insight into the relative ordering of biomarker trajectories (details in Supplemental data). Before fitting this model, the CSF and MRI biomarkers were Z-scored to young NCs (<40 years old, n=81, table 1). All statistical analyses were conducted in R (version 3.5.3) using the stats, emmeans. car, lmer, rstan and stanarm-packages [39].

3 Results

The presented analyses included 136 NCs and 219 MCs (age mean±SD 39±11; EYO mean±SD -9±11). In the MC group, 84 (39%) individuals had normal CSF ratio pTau/$A\beta_{42}$. Among MCs with an abnormal CSF pTau/$A\beta_{42}$ ratio, 64 (29%) individuals had CDR 0, 43 (20%) CDR 0.5 and 26 (12%) CDR 1-3. The group characteristics are shown in Table 1.

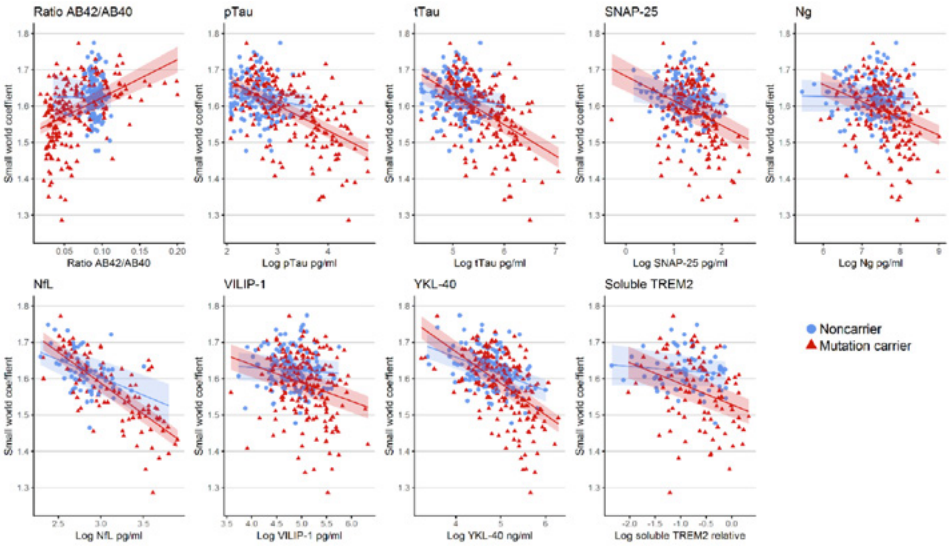

Figure 1 Associations between CSF biomarkers and grey matter networks for mutation carriers and noncarriers

Legend: Adjusted for sex. Prediction with 95% confidence intervals. sTREM2 = soluble TREM2 relative to a reference sample.

3.1 Associations between CSF biomarkers and the small world coefficient

Across the whole group, we found that all AD markers were related to alterations in grey matter networks (Table 2). Higher levels of NfL most strongly related to lower small world values (β±SE = -0.72±0.05; p<0.001), followed by YKL-40 (β±SE = -0.53±0.05; p<0.001), and pTau (β±SE = -0.53±0.05; p<0.001, Table 2). Models taking into account interaction terms of mutation status and CSF predictor, were significant for SNAP-25, Ng, pTau, tTau, NfL, VILIP-1 and YKL-40 (p<0.05, Fig. 1). Post-hoc comparisons showed that higher levels of SNAP-25 (-0.37 [CI 95%, -0.50,-0.24]) and Ng (-0.35 [CI 95%, -0.48,-0.21]), pTau (-0.58 [CI 95%, -0.69,-0.48]), tTau (-0.55 [CI 95%, -0.67,-0.44]) and VILIP-1 (-0.29 [CI 95%, -0.42,-0.16]) were related to lower small world values specifically in MCs. The association of higher NfL and YKL-40 and lower small world values was observed in both MCs and NCs, and this was stronger in MCs (NfL: MC = -0.76 [CI 95%, -0.89, -0.64] & NC = -0.44 [CI 95%, -0.77,-0.17]; YKL-40: MC = -0.61 [CI 95%, -0.72, -0.49] & NC = -0.32 [CI 95%, -0.48,-0.17]). When repeating models correcting for age, interaction effects for mutation status remained for SNAP-25, Ng, pTau, tTau, NfL and YKL-40 (p<0.05), but not for VILIP-1 (p=0.06). Next, we further studied in MCs whether the observed associations were specific to disease stage (Table S1, Fig. 2). No significant interaction terms with disease stage were observed, suggesting that associations of biomarkers and small world values were not specific to a certain stage.

Table 2 Associations between CSF markers and the small world coefficient

	Model 1 Predictor	Model 2 Mutation status* Predictor			Model 3 Mutation status* Predictor & adjustment for age		
Predictors	Predictor (beta)	Interaction (t)	Noncarriers (est slope)	Carriers (est slope)	Interaction (t)	Noncarriers (est adj slope)	Carriers (est adj slope)
$A\beta_{42/40}$ ratio	0.43 (0.05); p<0.001	1.8; p= 0.075	-0.01 (-0.47, 0.45); p=0.957	0.42 (0.31,0.52); p<0.001	1.9; p= 0.064	-0.18 (-0.57, 0.22); p=0.374	0.20 (0.11,0.30); p<0.001
pTau	-0.53 (0.05); p<0.001	-2.5; p= 0.014	-0.21 (-0.49, 0.07); p=0.144	-0.58 (-0.69, -0.48); p<0.001	-2; p= 0.047	0.06 (-0.18, 0.31); p=0.605	-0.39 (-0.49, -0.29); p<0.001
tTau	-0.48 (0.05); p<0.001	-3.1; p= 0.002	-0.16 (-0.38, 0.07); p=0.167	-0.55 (-0.67, -0.44); p<0.001	-2.6; p= 0.010	0.05 (-0.14, 0.24); p=0.596	-0.36 (-0.46, -0.26); p<0.001
SNAP-25	-0.33 (0.05); p<0.001	-2.1; p= 0.035	-0.13 (-0.31, 0.05); p=0.162	-0.37 (-0.50, -0.24); p<0.001	-2.2; p= 0.026	0.04 (-0.12, 0.19); p=0.645	-0.14 (-0.25, -0.02); p=0.019
Ng	-0.28 (0.05); p<0.001	-2.9; p= 0.004	-0.02 (-0.20, 0.16); p=0.836	-0.35 (-0.48, -0.21); p<0.001	-2.7; p= 0.008	0.07 (-0.07, 0.22); p=0.317	-0.20 (-0.31, -0.09); p=0.001
NfL	-0.72 (0.05); p<0.001	-2.2; p= 0.032	-0.44 (-0.71, -0.17); p=0.002	-0.76 (-0.89, -0.64); p<0.001	-3.7; P< 0.001	0.01 (-0.30, 0.32); p=0.940	-0.53 (-0.68, -0.38); p<0.001
VILIP-1	-0.26 (0.05); p<0.001	-2.0; p= 0.046	-0.05 (-0.24, 0.14); p=0.574	-0.29 (-0.42, -0.16); p<0.001	-1.9; p= 0.060	0.07 (-0.09, 0.22); p=0.401	-0.10 (-0.21, 0.01); p=0.078
YKL-40	-0.53 (0.05); p<0.001	-2.9; p= 0.004	-0.32 (-0.48, -0.17); p<0.001	-0.61 (-0.72, -0.49); p<0.001	-3.6; P< 0.001	0.05 (-0.11, 0.22); p=0.534	-0.30 (-0.43, -0.18); p<0.001
sTREM2	-0.33 (0.08); p<0.001	-2; p= 0.052	-0.08 (-0.33, 0.17); p=0.542	-0.40 (-0.61, -0.19); p<0.001	-1.3; p= 0.195	0.13 (-0.08, 0.34); p=0.229	-0.01 (-0.20, 0.18); p=0.912

Legend: All models were adjusted for sex; outcome = small world coefficient. sTREM2 = soluble TREM2 relative to a reference sample. All CSF markers, except the ratio are log-transformed.

3.2 Grey matter network and CSF biomarker trajectory by EYO

Finally, we estimated trajectories for all CSF and structural MRI markers according to EYO for the MCs, NCs, and the difference between MCs and NCs (Fig. 3; Table S2&3). Biomarker trajectories of the $A\beta_{42/40}$ ratio (EYO -18), $A\beta_{42}$ (EYO -16), pTau (EYO -18), tTau (EYO= -19), SNAP-25 (EYO= -15), Ng (EYO= -19) and VILIP-1 (EYO= -18) levels

were different in MCs as compared to NCs before differences were observed in grey matter networks (EYO= -8). NfL (EYO= -7) and YKL-40 (EYO= -7) trajectories were abnormal around the same time as grey matter networks, and sTREM2 (EYO= -3.5) and $A\beta_{40}$ (EYO= 0.5) showed abnormal levels in MCs compared to NCs later than grey matter networks.

4 Discussion

The main finding of our study is that CSF pathologic biomarkers showed associations with alterations in grey matter networks, and that axonal damage as measured with NfL showed the strongest relationship with worse grey matter network disruptions. Increased concentrations of the CSF markers for hyperphosphorylation of tau (pTau), neuronal injury and death (tTau and VILIP-1), and specific synaptic injury (SNAP-25 and Ng) were related to worse grey matter network organization in the MCs only. The observed associations were not dependent on staging based on a combination of the pTau/$A\beta_{42}$ ratio and the global CDR, suggesting that they were similar across disease development. According biomarker trajectories, most CSF markers showed abnormal levels before grey matter network abnormality in the MCs compared to the NCs, and for NfL and YKL-40 the timing was closest together with grey matter network alterations.

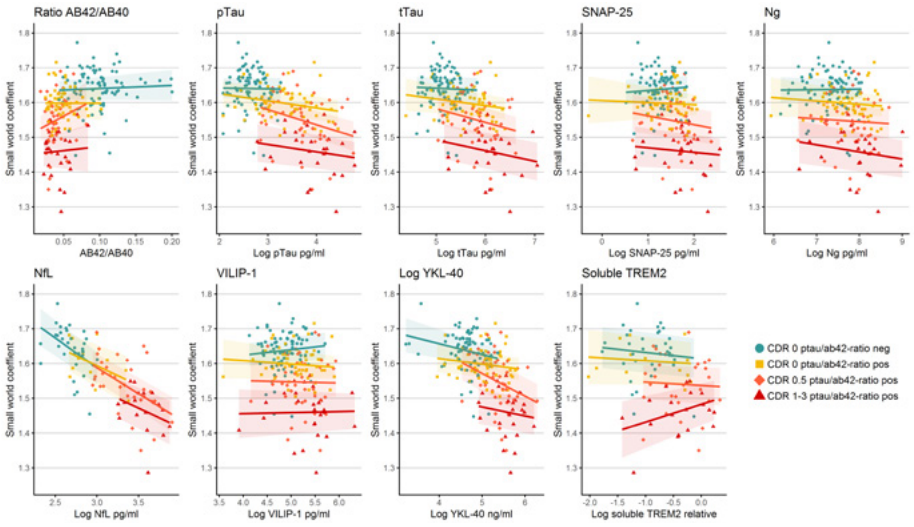

Figure 2 Associations between CSF biomarkers and grey matter networks within mutation carriers by disease stage

Legend: Adjusted for sex. Prediction with 95% confidence intervals. sTREM2 = soluble TREM2 relative to a reference sample.

So far, only the role of amyloid aggregation had been studied in relation to grey matter networks [14, 15]. Those findings suggested that grey matter networks are sensitive to brain structural changes related to amyloid aggregation in sporadic AD. Here, we found also that lower $A\beta_{42/40}$ ratios were associated with grey matter network disruptions. We further detected relationships between markers of other pathological processes in AD and grey matter network disruptions. The most pronounced association was observed for NfL, which suggests that loss of axonal integrity is an important factor for loss of grey matter network organization. The link between deterioration of grey matter covariance in AD to axonal tract damage supports the idea that grey matter covariance networks reflect, at least in part, axonal connectivity.

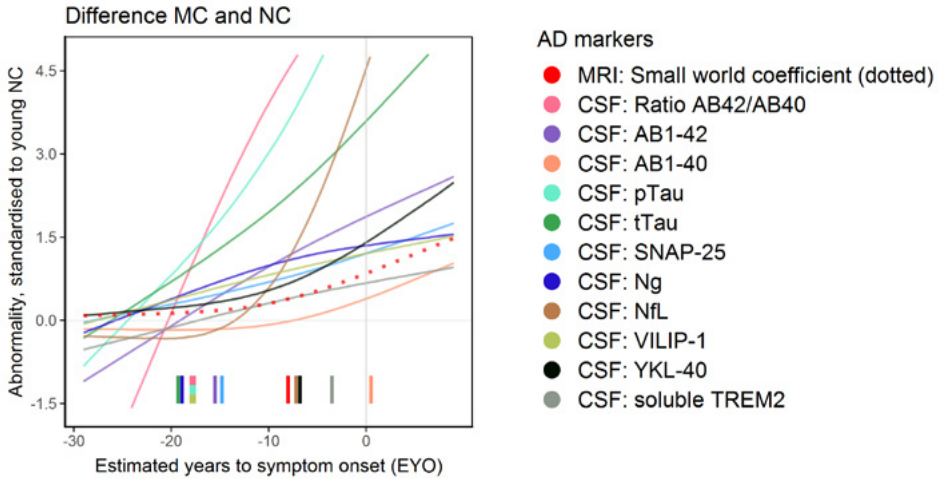

Figure 3 CSF and MRI biomarkers abnormality curves by EYO standardized to young noncarriers
Legend: The graphs show the median estimated curves standardized to the noncarriers mean and standard deviation (Table 1). All fitted lines are the median of the mixed models with a cubic spline, family random intercept and sex as covariate, and for the small world coefficient also total grey matter volume. These analyses depend on sample sizes, which were for: small world N=439; $A\beta_{42}$, $A\beta_{40}$, pTau, tTau N = 352; SNAP-25, VILIP1 N=330, Ng & YKL-40 N=331, sTREM2 N=218; NfL N = 210. The tickmarks are the point that the 99% credible intervals of the difference between mutation carriers and noncarriers is different than 0.

We also observed that higher levels of the synaptic markers (SNAP-25 and Ng), hyper-phosphorylation (pTau) and neuronal damage (tTau and VILIP-1) were associated with grey matter network disruption, and this was specific for MCs. Synaptic damage in neurodegeneration could possibly influence brain connectivity in the opposite way as during brain development, when synaptic maturation and co-activation play a role in increasing brain connectivity [3]. The biomarker trajectories suggest that synaptic damage and neuronal loss precedes the changes we observe with MRI, therefore MRI changes could be a downstream effect. Recent analyses had already demonstrated that CSF pTau and tTau increases very early in the course of ADAD, in a more parallel

fashion with amyloid aggregation than according to hypothetical models [28]. The findings suggest that loss of connectivity structures at the microscale, in neurons, could lead to disrupted connectivity of the brain as measured on MRI. Longitudinal studies are needed to further examine the temporal relationship of these processes in more detail.

The associations between increased NfL and the astrocyte marker YKL-40 and network disruptions were also observed in NCs. Previous studies have shown that during aging NfL and YKL-40 levels increase [19, 23, 40, 41], and grey matter networks measure decline [42], though less pronounced than in predementia AD. Our findings suggest that also in non-AD related aging, loss of axonal integrity and inflammation may impact on grey matter network integrity. A next step to disentangle whether these are pathological processes that may render the brain more vulnerable for neurodegeneration and possibly reflect cognitive decline in normal and/or non-AD related aging. sTREM2, released by microglia, fluctuates over the course of AD, with an increase close to symptom onset [24, 43]. sTREM2 levels showed a complex relationship with grey matter networks, as the association disappeared in mutation carriers when analyses were corrected for age. The trajectory curves for sTREM2 showed changes a few years later than for grey matter networks, thus the inflammatory process, reflected by sTREM2 increases may not be directly related to the brain structure changes as captured by the grey matter networks.

A strength is that we studied the pathophysiology over the full course of AD. Investigating ADAD MCs of the DIAN study, along with NCs, was a powerful way for a parallel investigation of multiple disease processes that may contribute to grey matter network disruption. Due to the causative genes, the cross-sectional trajectory can inform longitudinal changes. Still, the reality is more complex [44], meaning further study in a longitudinal design is needed to understand of the drivers and downstream effect in disease progression of AD. A shortcoming of fitting AD biomarker trajectories over the expected years to symptom onset is that results in part depend on sample sizes and model assumptions. Most EYOs of divergence were similar to previous studies, except for Ng and YKL-40, which is an indication of the level of robustness across modeling methods [23]. In addition, the exact meaning of the biomarkers levels is not fully understood, and we were unable to investigate brain tissue as part of this study. Another limitation is that we assessed linear relationships between CSF and grey matter network values, which may underestimate existing relationships. Therefore, we evaluated whether patterns depended on disease severity, which may give rise to non-linear patterns. Still, some of those disease stage groups were of small size, and larger samples are required to further investigate these relationships in detail. Lastly, we studied a primary summary measure of network organization, which was a way to reduce the number of comparisons and increase the interpretability. The findings warrant follow-up research to further investigate, whether associations are specific for specific brain areas and network measures.

To summarize, loss of synaptic integrity and in particular axonal integrity as measured with increased NfL in CSF seems to be related to disrupted grey matter

network organization in ADAD. These findings suggest that normalization of neuronal injury or synaptic processes might lead to stabilization or improvement of grey matter network integrity.

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Supplemental data Chapter 4.2

Methods and materials

Table S1 Association of CSF markers with the small world coefficient within MCs by severity groups

	Within MCs: interaction of group and CSF predictor				
Predictors	Interaction (F)	Ratio negative (est slope)	Ratio positive & CDR 0 (est slope)	Ratio positive & CDR 0.5 (est slope)	Ratio positive & CDR 1-3 (est slope)
$A\beta_{42/40}$ ratio	0.63; p=0.59	0.03 (-0.13,0.20); p=0.68	0.0 (-0.36,0.35); p>0.99	0.44 (-0.14,1.02); p=0.13	0.09 (-0.53,0.71); p=0.77
pTau	0.68; p=0.57	-0.02 (-0.41,0.37); p=0.92	-0.17 (-0.4,0.05); p=0.13	-0.36 (-0.67,-0.06); p=0.02	-0.18 (-0.54, 0.19); p=0.34
tTau	0.34; p=0.8	-0.04 (-0.36,0.27); p=0.78	-0.13 (-0.35,0.1); p=0.27	-0.25 (-0.56,0.05); p=0.10	-0.2 (-0.52,0.12); p=0.23
SNAP-25	0.36; p=0.78	0.06 (-0.19,0.31); p=0.64	-0.03 (-0.24,0.17); p=0.75	-0.14 (-0.43,0.16); p=0.36	-0.07 (-0.34,0.2); p=0.61
Neurogranin	0.24; p=0.87	0.01 (-0.21,0.23); p=0.92	-0.07 (-0.3,0.16); p=0.53	-0.06 (-0.36,0.24); p=0.69	-0.15 (-0.47, 0.17); p=0.35
NfL log	0.16; p=0.92	-0.77 (-1.36,-0.18); p=0.01	-0.51 (-1.17,0.14); p=0.12	-0.67 (-1.04,-0.3); p<0.01	-0.50 (-1.26, 0.26); p=0.2
VILIP-1	0.30; p=0.83	0.09 (-0.15,0.33); p=0.46	-0.06 (-0.26,0.15); p=0.58	-0.02 (-0.3,0.26); p=0.89	0.02 (-0.25, 0.28); p=0.9
YKL-40	0.75; p=0.52	-0.22 (-0.45,0.02); p=0.07	-0.11 (-0.35,0.13); p=0.37	-0.46 (-0.84,-0.07); p=0.02	-0.18 (-0.66,0.3); p=0.46
sTREM2	0.82 ; p=0.48	-0.13 (-0.57,0.32); p=0.58	-0.07 (-0.5,0.36); p=0.74	-0.07 (-0.52,0.38); p=0.76	0.36 (-0.15,0.86); p=0.17

Legend: All models were adjusted for sex; outcome = small world coefficient. sTREM2 = soluble TREM2 relative to a reference sample. All CSF markers, except the ratio are log-transformed.

Details statistical methods of biomarker trajectory model:
The statistical model to fit biomarker trajectories by EYO, described by (Gordon, Blazey et al. 2018), allowed for non-linear effects by using a restricted cubic spline to model EYO, with knots on the 0.1, 0.5 and 0.9 of the distribution. The models had fixed terms for EYO, mutation status, their interaction and a random effect for family cluster. Models were adjusted for sex, and for the small world coefficient additionally for total grey matter volume. For the trajectories we used the biomarker data of the first available visit (Table S1 below). Model parameters were estimated with Hamiltonian Markov chain Monte Carlo sampling of the posterior distribution, with cauchy prior, 10,000 iterations in 8 chains, and thinning of 10 in the STAN and rstanarm package for R (Carpenter, Gelman et al. 2017). The EYO point of divergence is when the 99% credible intervals of the difference distribution between MCs and NCs did not overlap 0. We also provide the 95% and 99.5% of the credible intervals (Table S2).

R code: model_fit <- stan_glmer(standardized_biomarker_value ~ (1 | family_id) + eyo_1 + eyo_2 + mutation_status + eyo_term_1*mutation_status + eyo_term_2*mutation_status + sex, data = data, family = gaussian(), prior = cauchy(), prior_intercept = cauchy(), chains = 8, cores = 1, iter = 10000, thin = 10)

Table S2 Baseline values for biomarkers used for EYO in comparison to crossmodal data

	Noncarriers (NCs)	Noncarriers (NCs)	Mutation carriers (MCs)	Mutation carriers (MCs)
Grey matter network				
Small world coefficient	1.63 ± 0.05	1.62 ± 0.05	1.59 ± 0.09	1.59 ± 0.08
Traditional CSF markers				
$A\beta_{42}$ pg/ml	1,379 ± 464	1,407 ± 466	951 ± 635	974 ± 634
$A\beta_{40}$ pg/ml	15,491 ± 4490	15,698 ± 4418	1,4763 ± 4851	14,862 ± 4760
pTau pg/ml	14 ± 5	14 ± 5	32 ± 24	31 ± 23
tTau pg/ml	168 ± 56	169 ± 55	295 ± 169	290 ± 162
Ratio $a\beta_{42/40}$	0.088 ± 0.010	0.089 ± 0.010	0.065 ± 0.034	0.066 ± 0.035
Emerging CSF markers				
SNAP-25 pg/ml	3.6 ± 1.3	3.6 ± 1.3	4.6 ± 1.9	4.6 ± 1.9
Ng pg/ml	1,529 ± 736	1,563 ± 741	2303 ± 1186	2,297 ± 1,212
NfL pg/ml	820 ± 622	793 ± 544	1925 ± 1900	1,939 ± 1,762
VILIP-1 pg/ml	132 ± 52	133 ± 50	176 ± 78	174 ± 79
YKL-40 ng/ml	135 ± 66	133 ± 66	178 ± 92	173 ± 88
sTREM2, relative to reference sample	0.48 ± 0.22	0.47 ± 0.22	0.59 ± 0.29	0.58 ± 0.29

Legend: Light grey color are the values of table 1 for the main analysis of crossmodal comparison

Table S3 Estimated years to onset of divergence between mutation carriers and noncarriers

	EYO of divergence according 99% credible interval	EYO of divergence according 95% credible interval	EYO of divergence, according 99.5% credible interval
Grey matter network			
Small world coefficient	-8	-10.4	-7.5
Traditional CSF markers			
$A\beta_{42/40}$ ratio	-17.8	-18.4	-17.6
$A\beta_{42}$	-15.5	-16.4	-15.2
$A\beta_{40}$	0.5	-1	1.2
pTau	-17.7	-19.1	-17.2
tTau	-19.2	-20.4	-18.4
Emerging CSF markers			
SNAP-25	-14.8	-17.9	-12.6
Ng	-19	-20.1	-18.2
NfL	-7	-8.1	-6.7
VILIP-1	-17.9	-19.8	-16.8
YKL-40	-7	-10.1	-6
sTREM2	-3.5	-6.7	-2.2

Legend: These analysis depend on sample sizes, which were for: small world N=439; Aβ42, Aβ40, pTau, & tTau N = 352; SNAP-25 & VILIP1 N=330, Ng & YKL-40 N=331, sTREM2 N=218; NfL N = 210.

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5

Chapter 5

Summary and general discussion

Secondary prevention trials in Alzheimer disease (AD) aim to delay, or even prevent, the onset of dementia. Most novel, and challenging, are clinical trials intervening in the preclinical stage, when disease signs are subtle and outcome measures have been shown to have insufficient sensitivity. How to optimally design and conduct these types of trials is thus a timely topic of scientific debate. Our incomplete insight in the natural course of pre-dementia AD (preclinical + prodromal) complicates creating appropriate selection criteria and outcomes to measure effectiveness. In addition, a good enrolment rate is essential, but it is not yet established how to find and screen these potential participants, who present in insufficient numbers in memory clinics. This thesis contains several studies relevant for secondary prevention of AD. We estimated the duration of preclinical, prodromal, and dementia stages of AD (Ch. 2.1,2.2); studied strategies for recruitment and selection of participants for secondary prevention studies (Ch. 3.1,3.2); and investigated grey matter network disruption as a potential outcome measure (Ch. 4.1,4.2).

The main findings are:

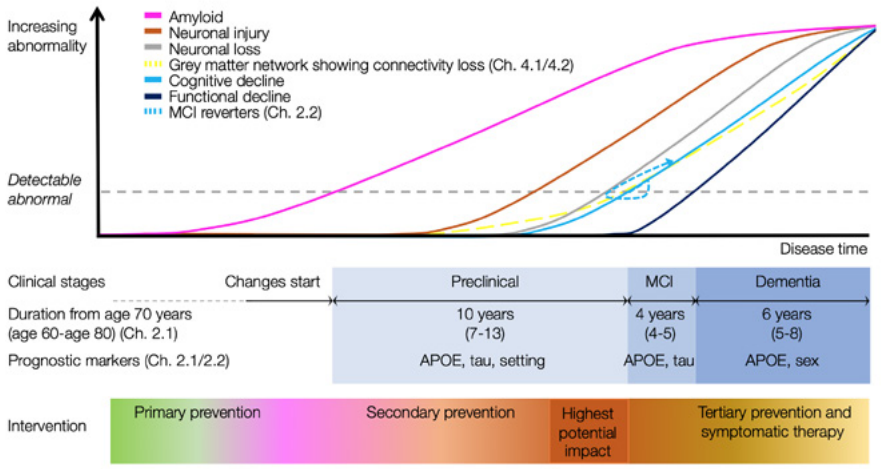

Figure 1 Thesis results incorporated in AD progression model with prevention strategy
Adapted from Jack et al. 2013 [49].

(1) Clinical course of AD

- Total AD duration varied between 24 years for an individual aged 60, and 15 years for an individual aged 80.
- For an individual aged 70 with preclinical AD, estimated duration of preclinical AD was 10 years, of prodromal AD 4 years, and of dementia 6 years.
- The duration of AD stages is dependent on age, setting, sex, *APOE*, and CSF tau.

(2) Recruitment for Alzheimer disease research

- We set up the virtual EPAD Registry to show the feasibility to preselect individuals from ongoing studies for future AD prevention studies.
- Lower age, higher education, male sex, and a family history of dementia were associated with an increased willingness and ability to participate in future AD prevention studies.
- Higher age and *APOE* ε4 carriership was associated with an increased risk for amyloid pathology.

(3) Grey matter network analysis is a potential surrogate endpoint for trials

- Individuals who carry an autosomal dominant AD mutation show increased decline over time in grey matter connectivity 6 years before the estimated time of symptom onset.
- Loss of grey matter connectivity correlates with cognitive decline.
- Loss of grey matter connectivity was associated with CSF markers of tau, synaptic and axonal degeneration, and astrocyte activation.

This chapter has the following structure: (1) a summary of the studies with context, (2) relevance and implications of the findings for trials and future treatment, (3) methodological considerations, and (4) future directions for clinical research on secondary prevention of AD and conclusion.

1 Summary of the thesis and context

1.1 Clinical course of AD

Duration of clinical AD stages and prognostic factors

In Chapter 2.1, we estimated duration of the preclinical (amyloid-positive normal cognition), prodromal (amyloid-positive MCI), and dementia stages of AD according the individuals' age, sex, *APOE* genotype, CSF tau levels and the setting (clinic or research). The estimates were based on multi-cohort data of 3,268 individuals. The overall duration of AD from amyloid-positivity ranged from 24 years at age 60 to 15 years at age 80. The estimates for an individual with preclinical AD, aged 70, were 10 years in the preclinical AD stage, 4 years in the prodromal AD stage, and 6 years in the dementia stages. Comparable to our study, one previous study estimated that pre-dementia AD is 17 years based on amyloid accumulation rates [1]. Lower age-specific durations of preclinical and prodromal AD were driven by higher mortality, faster decline, and lower reversion from MCI. This is in concordance with an exponential increase in AD dementia prevalence with age [2]. Higher progression rates at higher ages may be driven by a longer exposure duration at baseline or lower resilience [3].

We found a pronounced effect of cohort: for example preclinical AD, at age 70, had in a research setting a duration of 11 years, which was almost 3 times larger than the duration in clinical setting of 4 years. The shorter pre-dementia stages in the memory clinic patients compared to research participants may have two reasons. Individuals are longer in that stage at entry or those with more aggressive disease present more frequent at memory clinics. Compared to non-carriers, *APOE* ε4 carriers had a shorter duration of preclinical (~ -1.5 to -4 years) and prodromal AD (~-1 year). The shorter pre-dementia duration in *APOE* ε4 carriers is in line previous studies showing higher amyloid accumulation rates and an earlier dementia onset in *APOE* ε4 carriers [4-6]. When CSF tau was abnormal at baseline, preclinical AD was ~3 years and prodromal AD ~2.5 years shorter. The shortened preclinical and prodromal AD stages for individuals with elevated CSF tau levels at baseline is also in accordance with many studies showing to faster cognitive decline and neurodegeneration in those groups [7-10]. In conclusion, the estimations of AD duration improve when age, sex, *APOE* genotype, tau and setting are taken into account.

MCI reverters

Not all individuals with MCI progress to dementia. Ten to 30 percent of individuals with MCI show improvement to normal cognition [11], which seems a positive event. However, others had shown that MCI reverters remain at increased risk for dementia [12, 13]. We postulated this increased risk could be due to underlying AD.

In Chapter 2.2, we investigated which baseline factors, i.e., demographics, cognition, CSF and imaging markers, were associated with decline to MCI or dementia after initial reversion. We selected two independent samples of MCI reverters, the Alzheimer Disease Neuroimaging Initiative (ADNI) and Amsterdam Dementia Cohort. We found that the MCI reverters who subsequently showed decline were older and had abnormal amyloid PET and CSF tau levels more often compared to those who remained normal. In this clinically diverting group, AD biomarkers aid in distinguishing, a good prognosis, the stable MCI reverters, from those that are likely to decline again.

1.2 Strategies for recruitment and prescreening for studies on prevention of AD dementia

Trials for a secondary prevention strategy for AD have started to involve the search for individuals with normal cognition and evidence of amyloid pathology (EARLY and A4 trial) [14], or genetic risk factors, *APOE* ε4 and/or TOMM40 (TOMMORROW and Generation I&II trial). As individuals from the general population have a relatively low prevalence of amyloid accumulation or AD risk alleles as well as contra-indications for trial participation, these studies are facing high (pre)screen failure rates [15]. In the A4 trial, the total screen failure rate was 83% [16]. We set up the EPAD Registry, as an alternative to memory clinic referrals, outreach and advertisement. The aim was to facilitate recruitment and reduce screen failure for the EPAD longitudinal cohort study by enabling prescreening of individuals from existing studies. A subset of the EPAD participants will enroll in future clinical trials, thus general contra-indications for trials are checked, but amyloid-positivity is not required to pass the screening (Chapter 3.1). We compared the enrolment from 4 settings (memory clinic, general population, online and in-person volunteers research). Participation rates were highest in the memory clinic (59%) and lowest in the population-based cohort (3%). Despite the difference in participation rates, the total recruitment numbers were similar between settings as cohorts with a low participation rate had the largest number of participants in our study.

The percentages amyloid-positive individuals were around 30% in both the A4 trial screening and the EPAD trial-ready cohort participants. In Chapter 3.2, we assessed whether the presence of AD risk factors influenced participation rates and prevalence of amyloid positivity across the different settings of the recruitment sources. We found that individuals who were relatively young, had a higher education, male sex, and a family history of dementia were more often willing and able to participate in the EPAD trial-ready cohort. Among those who enrolled in the EPAD trial-ready cohort, the prevalence of amyloid positivity was higher for those who were at baseline relatively old and those carrying the *APOE* ε4 allele. These predictors were robust across settings.

1.3 Grey matter networks as potential surrogate endpoint for trials

Grey matter network changes align with progression in autosomal dominant AD

For a reliable measurement of treatment effects in secondary prevention, we need outcome measures that can track change in pre-dementia stages. In chapter 4.1, we studied a novel marker of AD progression, i.e., grey matter network measures. We tested whether, and how, such networks declined over the disease course in individuals carrying an autosomal dominant AD (ADAD) mutation compared to their noncarrier family members, as a function of estimated years to symptom onset. Mutation carriers had an increased rate of decline of the global grey matter network measures from 6 years before symptom onset. This was the first study to show that network disruptions decline within individuals with AD over time. The speed of grey matter network decline was predicted by the rate of amyloid accumulation, and closely associated to other markers of neurodegeneration on MR, FDG-PET and to cognitive decline. It provides a validation of grey matter network measures as a marker for disease progression in AD.

What are the biological correlates of grey matter networks disruption in AD?

In chapter 4.2, we further investigated the biological mechanisms underlying grey matter network disruptions in ADAD. To this end, we studied how grey matter network disruptions related to pathological markers in CSF that are known to be involved in AD, covering amyloid and tau aggregation, neuronal death, synaptic and axonal injury, and inflammation. We found that more abnormal levels of the CSF markers correlated with network disruptions. For elevated levels of markers of synaptic injury, tau, and neuronal death the associations with network disruption were specific for the mutation carriers, while the associations were also present in the noncarriers for axonal injury (NfL) and astrocyte activation. Higher NfL levels were most strongly associated with disrupted networks, which supports that axonal integrity plays a role in grey matter networks [17]. When comparing biomarker trajectories by the estimated years to symptom onset, we found that amyloid, tau, synaptic, and neuronal death markers diverged between the mutation carriers and noncarriers before, and axonal injury and astrocyte activation around the same time as grey matter network measures. The findings suggest that grey matter network disruptions may reflect loss of axonal connectivity in AD, occurring downstream from synaptic and neuronal injury.

2 Relevance of the findings for design of secondary prevention studies

2.1 Implications for trial design and inclusion criteria

Our finding that the pre-dementia period was 12 to 17 years has several implications. Trials in younger subjects with preclinical AD would take 15-20 years before the effect on progression to dementia can be assessed, which may make prevention trials challenging. A solution for this problem is to use surrogate endpoints [18]. The A4 and EARLY trial have a cognitive composite, but surrogate endpoints could also be biomarkers of disease progression, such as connectivity loss. When the disease

trajectory is well established, health economic models, taking into account age and setting, can extrapolate treatment effects, as measured at intermediate time points to estimate outcomes relevant for patients and/or society. In addition, the prognostic information on amyloid-positivity and MCI reversion forms an improvement for the previously available information for (potential) trial participants.

Enrichment and stratification with *APOE* and tau within amyloid-positive individuals

The pre-dementia period was influenced by age, *APOE*, CSF tau, meaning that further stratification by these factors could increase the power of secondary prevention trials. Enrichment for the *APOE* ε4 allele may result in more short-term progression and faster decline [4]. An advantage of stratification by *APOE* ε4 is that it increases the etiological and phenotypical homogeneity of the sample. However 40% of individuals with AD do not carry this allele and a treatment is also needed for them [19]. Enrichment with abnormal CSF tau would also increase the power to detect clinical and cognitive decline in amyloid-positive individuals without dementia [9]. Moreover, individuals who improve spontaneously are detrimental for the power of trial. Individuals with amyloid and tau-positive MCI rarely revert to normal cognition, and if so, are at increased risk to decline again. Of note, enrichment by markers related to fast decline will not necessarily increase the likelihood for trial success, in case an enrichment marker negatively affects the treatment response. For example: while a decreased cortical thickness is prognostic for faster cognitive decline, individuals with more atrophy at baseline could respond worse to a specific treatment. Therefore, it is important to take hypotheses on the relation between enrichment factors and the mode of action of the compound into consideration for the design of trial.

Detection of late-stage preclinical AD

We found that within amyloid-positive individuals a clinical visit, generally prompted by complaints of the patient or their relatives, is a strong prognostic factor for clinical progression. The finding suggests that these individuals are in late-stage preclinical AD, which would be a window of high potential impact of a preventive treatment (Figure 1). It also supports the pursuit to delineate which subjective signs and complaints reflect very early clinical progression of AD [20-22] in order to refine selection of individuals who may have clinical benefit from a treatment.

2.2 Implications for recruitment and prescreening for studies on prevention of AD dementia

Registries for participant selection and engagement

The EPAD Registry approach successfully kick-started enrolment for the project, with low screen failure due to contra-indications. However, we noted an issue with sustainability, as existing cohorts became depleted if not continuously enrolling new participants. A registry with continued enrollment, with a wider purpose seems more sustainable; either facilitating more studies and/or including data collection. In the

Netherlands, we performed a small pilot, modelled after the Brain Health Registry in USA [23]. The mature version of this participant registry was launched launched in 2019 [Zwan et al. in preparation]. Initiatives with related scopes include: TrialMatch (USA), JoinDementiaResearchUK (UK), and StepUP (Australia). We found that the set-up and maintenance require expertise in AD, online recruitment and engagement, and technological aspects. A generic registry is thus associated with substantial costs, but reduces the recruitment efforts in other projects, and probably even improves the percentage of studies with successfully completed enrolment. Nonetheless, it may be difficult to prove efficacy of registries, as recruitment effort and time tend to be underestimated [15].

Implementation of strategies for trial screening

Another implication of our studies on recruitment and screening was that currently available predictors for amyloid positivity have a modest predictive value. To obtain lower screen failure rates based on amyloid negativity, the population that qualifies for screening should to be massively restricted. If additional selection criteria beyond amyloid-positivity were to be included, as suggested in previous paragraphs, the prevalence of eligible individuals will be even lower, which proportionally increases the recruitment challenge [24]. A powerful way to decrease the screening burden is the commonly applied step-wise screening approach. In light of the recent developments in blood tests for Abeta and neurodegeneration [25-28], a blood test as a first step during screening could reduce the number of PET scans or CSF collections. A potential advantage of using a biological state marker, rather than a risk factor such as family history for dementia, is that larger proportion of individuals who may qualify can have access to the study screening. When these participants are drawn from a participant registry, and they subsequently screen fail for one study, the collected information can be (re)used for prescreening in future studies. Participants can then apply for re-screening, when a biomarker retest is sensible, after comorbidities have resolved, or personal circumstances have changed. In addition, the registry can enable the participants to share their data with multiple scientists, minimizing tedious repetition for the participant.

2.3 Implications for grey matter networks as surrogate endpoint

The investigation of grey matter networks in ADAD showed with respect to the potential use as a surrogate endpoint for trials that the networks decline within individuals over time in AD. However, most of the pure extracted measures, network degree, connectivity density and path length showed large variations within individuals over time. This intra-individual variability limits its use as an endpoint in clinical trials. In contrast, normalization to a reference network seemed to increase the ability to track change over time. Therefore, these small world measures are better suited as potential endpoints. Grey matter networks measures predicted future cognitive decline and neurodegeneration, which suggests that reduced decline of the grey matter

connectivity, or even improvement, may point towards robust disease modification. Our investigations should be extended by power calculations, as well as testing which of network metric(s) is superior and whether network measures have added value compared to current surrogate outcomes on cognition. It may also be possible to identify an optimal combination of structural grey matter markers with increased statistical power to detect change over time.

3 Methodological considerations

3.1 Staging and duration of the disease course of AD

A problem of studying a slowly progressive disease as AD is that the 'exposure duration' differs between individuals at study entry. The rate and the degree of preceded brain damage are unknown, while these influence the speed of progression [29]. Staging models intent to align individuals better on the disease severity [30-35]. The assumption is that when the staging within preclinical AD is more precise, disease-related abnormalities stand out. For all new modeling approaches, the balance between identification of plausible, relevant patterns, without over-specification towards the hypothesis presents a challenge. This is for example a risk when we include variables that are also part of the diagnostic criteria for MCI and dementia as predictors in classification modeling. In our ADAD project, we used the mutation-specific age at dementia onset as a surrogate timeline [36]. For interpreting an EYO of divergence between mutation carriers and noncarriers, it is important keep in mind that this time point is influenced by sample size, model specifications, the exact definition of EYO, as well as between subject variability, floor- and ceiling-effects of the investigated disease markers. In addition, longitudinal analyses do not always overlap completely with the cross-sectional trajectories [37]. In our study on grey matter networks decline over time was detectable later, likely due to a lower sample size. Still, the shape of the curves overlapped, pointing consistently towards an accelerating rate of decline of grey matter networks over the disease course.

In chapter 2.1, we used short-term longitudinal data of amyloid-positive individuals to estimate the AD clinical stage durations [38, 39]. Here, it is also important to remember the assumptions made. An assumption in our MSM model was that we presume that everyone who is amyloid-positive is on a trajectory to AD dementia. A limitation was that we could not include a separate tau stage in preclinical AD, due to few repeated measurements of tau. Mortality risk can be accounted for by multi-state or competitive risk models, but has mostly been ignored in AD studies. The primary reason for not incorporating mortality in prognostic studies with biomarkers is often because it is simply not systematically checked after attrition or completion of study visits. This was also a limitation in our study, and may be an explanation for the low mortality proportion in the preclinical AD group. Repeating this analysis with longer follow-up until death would improve the accuracy, and may allow further refinement of the model with additional covariates or stages.

3.2 Study population

We found low MCI reversion rates in the memory clinic cohort, and a shorter duration of pre-dementia stages in a clinical compared a research setting. This is in concordance with setting effects on the incidence of MCI and dementia, in amyloid biomarkers confirmed individuals with normal cognition or MCI, respectively [10, 40, 41]. Improved phenotyping may lead to better alignment between different cohort types, but at this stage, the different absolute risk estimates across populations warrants cautiousness when extrapolating results, for example in economic models.

We also studied the ADAD population. By definition, ADAD and sporadic AD differ in genetic causes, leading to questions on the generalizability of findings in ADAD. An advantage of studying the development of AD in mutation carriers of ADAD is the limited aging effects, due to the relatively young age of dementia onset. Grey matter network disruptions were consistent between the forms of AD. With regards to treatment development it is critical to learn whether causative cascades between these forms of AD converge, and based on our findings this convergence is likely to be upstream from grey matter network disruptions.

4 Future perspectives and conclusion

Studying the disease course of AD

Utilizing the larger datasets and increasingly follow-up durations, researchers started to apply more advanced methods to better understand progression of AD in the pre-dementia stages. Yet, repeated biomarkers measurements over long time periods, and observations of biomarker transitions at still rare [32, 42]. Information on biomarkers during early and mid-life is also sparse, though important, because early-life changes or a disequilibrium from an early-life homeostasis may relate to the development of AD in late-life. Another restriction in the advancement of prevention trial design are challenges with regards to the markers available for the disease monitoring. First, while most markers have a good diagnostic value, most are less suitable for predicting and monitoring disease progression. As it is unlikely that we can find one perfect disease marker for progression, the development of a practical toolbox seems more realistic, to which grey matter network measure can be added. Secondly, aging individuals can have multiple pathologies contributing to the speed of decline. Therefore, good markers for the other pathologies are important for AD modeling, to enable accounting for other factors. With a precise individual prognosis, we would be able to offer a future treatment to the appropriate persons at the right disease stage [43].

Run-in data for selection and treatment evaluation in trials

With regards to treatment evaluation, a run-in period (without any treatment) has been shown to have the potential to increase the power over cross-sectional baseline values [44, 45]. A run-in period is already implemented in DIAN-TU and the EPAD project to reduce the number of participants needed. Future trials should continue to refine and optimize the use of a run-in period.

Selection criteria intent to restrict the inclusion to individuals with potential benefit from a treatment [43]. While selection criteria on clinical, cognitive and biological signs with cross-sectional cut-offs are practical, the premorbid levels of those markers differ between individuals. Therefore, a cross-sectional value within the normal range does not exclude decline from the premorbid levels. In future trials, longitudinal inclusion criteria may facilitate selection of the appropriate individuals. Further investigations should clarify the pros and cons of essentially restricting enrolment to those who demonstrated decline (or no improvement) over time on specific markers before the start of trial.

Research participants motivation and engagement, why and how?

Prevention trial participation will not fit everyone's personal circumstances, life style and personality. Motivations include aspects of: having an affected family member or partner, altruism, help the next generation, passion for science, worries about cognition, curiosity about their body, meaningful activity, prospect of frequent check-ups, or hope for personal benefit. The population in clinical trials in Europe and the USA, and also in EPAD, is very homogenous Caucasian and higher-than-average educated. While upholding the appreciation for those participating, it would be better for the generalizability and recruitment rates if clinical trial populations had more diverse backgrounds. Increasing diversity requires specific adaptations to the trial design and recruitment strategy [46]. Interaction with the new type of research participants can teach us what drives individuals to join AD studies and which practical aspects of clinical trials may hamper participation. An alternative way to increase the recruitment (and retention) may be to lower the burden for participants [47]. This could include for example to develop cognitive tests that are less boring to complete, or replacing site visits by teleconferencing or home visits. Another practicality is the requirement for an informant about the participants daily functioning, which can preclude (trial) participation and cause attrition. Possibly the development of clinical trial robots, similar to care robots [48], could offer an alternative to a human informant for the trial, and by home observations reduce the number of tests and site visits needed.

4.1 Conclusion

In this thesis, we have investigated the trajectory of AD with different methods and figure 1 places these findings in the context of AD pathological cascade. This knowledge is important for understanding the development of AD, how to structure future trials in different stages, as well as for the implementation of treatments when these become available. Most previous secondary prevention trials targed amyloid, also the focus in our studies. Relatively new is that novel leads are more diverse and now include anti-tau compounds. Therefore, the maturation of participant registries and better blood-based screening markers will allow flexibility for adaptions in selection criteria. With our modern tools, tireless efforts of researchers and participants, and inspired by recent treatment successes in neurological diseases, a break-though could be around the corner. When this will happen is a matter of speculation.

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APPENDIX

APPENDIX

List of publications

Vermunt, L., Muniz-Terrera, G., ter Meulen, L., Veal, C., Blennow, K., Campbell, A., Carrié, I., Delrieu, J., Fauria, K., Huesa Rodríguez, G., Ingala, S., Jenkins, N., Molinuevo, J.L., Ousset, P.J., Porteous, D., Prins, N.D., Solomon, A., Tom, B.D., Zetterberg, H., Zwan, M., Ritchie, C.W., Scheltens, P., Luscan, G., Brookes, A.J. and Visser, P.J., for the IMI-EPAD collaborators. (2020). **Prescreening for European Prevention of Alzheimer Dementia (EPAD) Trial-Ready Cohort: Impact of AD risk factors and recruitment settings.** *Alzheimer's Research & Therapy*, 12(1).

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List of PhD theses of Alzheimer Center Amsterdam

1. L. Gootjes: Dichotic Listening, hemispheral connectivity and dementia (14.09.2004)
2. K. van Dijk: Peripheral nerve stimulation in Alzheimer's disease (16.01.2005)
3. R. Goekoop: Functional MRI of cholinergic transmission (16.01.2006)
4. R. Lazeron: Cognitive aspects in Multiple Sclerosis (03.07. 2006)
5. N. Schoonenboom: CSF markers in dementia (10.11.2006)
6. E. Korf: Medial temporal lobe atrophy on MRI – risk factors and predictive value (22.11.2006)
7. B. van Harten: Aspects of subcortical vascular ischemic disease (22.12.2006)
8. B. Jones: Cingular cortex networks – role in learning and memory and Alzheimer's disease related changes (23.03.2007)
9. L. van de Pol: Hippocampal atrophy from aging to dementia – a clinical and radiological perspective (11.05.2007)
10. Y. Pijnenburg: Frontotemporal dementia – towards an earlier diagnosis (05.07.2007)
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12. E. van Straaten: Vascular dementia (11.01.2008)
13. R. Vogels: Cognitive impairment in heart failure (11.04.2008)
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15. G. Karas: Computational neuro-anatomy (19.06.2008)
16. F. Bouwman: Biomarkers in dementia – longitudinal aspects (20.06.2008)
17. A. Gouw: Cerebral small vessel disease on MRI – clinical impact and underlying pathology (20.03.2009)
18. H. van der Roest: Care needs in dementia and interactive digital information provisioning (12.10.2009)
19. C. Mulder: CSF Biomarkers in Alzheimer's disease (11.11.2009)
20. W. Henneman: Advances in hippocampal atrophy measurement in dementia – beyond diagnostics (27.11.2009)
21. S. Staekenborg: From normal aging to dementia – risk factors and clinical findings in relation to vascular changes on brain MRI (23.12.2009)
22. N. Tolboom: Imaging Alzheimer's disease pathology in vivo – towards an early diagnosis (12.02.2010)
23. E. Altena: Mapping insomnia – brain structure, function and sleep intervention (17.03.2010)
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26. J. Sluimer: Longitudinal changes in the brain (28.04.2011)
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31. W. de Haan: In a network state of mind (02.11.2012 – cum laude)
32. D. van Assema: Blood-brain barrier P-glycoprotein function in ageing and Alzheimer's disease (07.12.2012)
33. J. Goos: Cerebral microbleeds – connecting the dots (06.02.2013)
34. R. Ossenkoppele: Alzheimer PEThology (08.05.2013)
35. H. Jochemsen: Brain under pressure – influences of blood pressure and angiotensin-converting enzyme on the brain (04.10.2013)
36. A. van der Vlies: Cognitive profiles in Alzheimer's disease – recognizing its many faces (27.11.2013)
37. I. van Rossum: Diagnosis and prognosis of Alzheimer's disease in subjects with mild cognitive impairment (28.11.2013)

38. E. Møst: Circadian rhythm deterioration in early Alzheimer's disease and the preventative effect of light (03.12.2013)
39. M. Binnewijzend: Functional and perfusion MRI in dementia (21.03.2014)
40. H. de Waal: Understanding heterogeneity in Alzheimer's disease – a neurophysiological perspective (25.04.2014)
41. W. Jongbloed: Neurodegeneration – biochemical signals from the brain (08.05.2014)
42. E. Koedam: Early-onset dementia – unraveling the clinical phenotypes (28.05.2014)
43. A. van Harten: The road less traveled: CSF biomarkers for Alzheimer's disease – predicting earliest cognitive decline and exploring microRNA as a novel biomarker source (07.11.2014)
44. A. Hooghiemstra: Early-onset dementia – with exercise in mind (03.12.2014)
45. L. Sandberg-Smits: A cognitive perspective on clinical manifestations of Alzheimer's disease (20.03.2015)
46. F. Duits: Biomarkers for Alzheimer's disease – current practice and new perspectives (01.04.2015)
47. S. Adriaanse: Integrating functional and molecular imaging in Alzheimer's disease (07.04.2015)
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62. A. Leeuwis: Connecting heart and brain – vascular determinants of cognitive impairment and depressive symptoms (02.07.2019)
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64. A. van Loenhoud: Cognitive reserve in Alzheimer's disease – a perspective on the flourishing and withering brain (18.09.2019)
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69. L. Vermunt: Secondary prevention for Alzheimer disease - timing, selection, and endpoint of clinical trials (13.03.2020)

List of abbreviations

Aβ	Amyloid beta
AD	Alzheimer disease
ADAD	Autosomal dominant inherited Alzheimer disease
APOE	*Apolipoprotein E*
APP	*Amyloid precursor protein*
CDR	Clinical Dementia Rating scale
CDR-SOB	Clinical Dementia Rating scale - sum of boxes
CI	Confidence interval OR credible interval
CSF	Cerebrospinal fluid
DIAN	Dominantly Inherited Alzheimer Network
DIAN-Obs	Dominantly Inherited Alzheimer Network Observational cohort study
DIAN-TU	Dominantly Inherited Alzheimer Network-Trials Unit (clinical trial platform)
EPAD	European Prevention of Alzheimer's Dementia
EPAD-LCS	European Prevention of Alzheimer's Dementia - Longitudinal cohort study
EPAD-PoC	European Prevention of Alzheimer's Dementia - Proof of concept trial
EYO	Estimated years to symptom onset
FDG	18F-Fluorodeoxyglucose
FU	Follow-up
GM	grey matter
GS	Generation Scotland
MCI	Mild cognitive impairment
MMSE	Mini-mental state examination
MRI	Magnetic resonance imaging
MSM	Multi-state model
MTA	Medial temporal lobe atrophy
MC	Mutation carrier
NC	normal cognition OR noncarrier
NfL	Neurofilament light chain
Ng	Neurogranin
NNS	Number needed to screen
NNPS	Number needed to prescreen
PSEN1/2	*Presenilin 1/2*
P value	Probability value (probability of obtaining results at least as extreme as observed, assuming a correct null hypothesis.)
PET	Positron emission tomography
pTau	Phosphorylated tau
ROI	Region of interest (of the brain on a scan)
SCD	Subjective cognitive decline
SD	Standard deviation
SNAP-25	synaptosomal-associated protein-25
SUVR	Standardized uptake value ratio
sTREM2	soluble TREM2, a microglia marker
tTau	Total tau
VILIP-1	Visinin-like protein 1
WMH	White matter hyperintensities
YKL-40	Chitinase-3-like protein 1

Nederlandstalige samenvatting

Doel van het proefschrift

In dit proefschrift maken we gebruik van biomarkers en klinische metingen om nieuwe inzichten te genereren voor de opzet en uitvoering van secundaire preventiestrategieën ter voorkoming van dementie door de ziekte van Alzheimer. We gebruiken onderzoeksgegevens van verschillende internationale cohortonderzoeken van de deelnemers met en zonder dementie om het klinisch beloop van de ziekte van Alzheimer beter te begrijpen. We schatten de duur en beïnvloedende factoren van het preklinische (geen cognitieve stoornissen), prodromale (milde cognitieve stoornissen [MCI]), en dementie-stadium van de ziekte van Alzheimer (hoofdstuk 2.1,2.2). We presenteren strategieën voor werving en selectie van deelnemers voor secundair preventie- en cohortonderzoek met als voorbeeld het EPAD-project (hoofdstuk 3.1,3.2). Het grijze-stofnetwerk is een hersenmaat berekend op structurele MRI, die de kwaliteit van de samenwerking van hersenonderdelen representeert. In hoofdstuk 4 onderzoeken we verstoring van dit hersennetwerk bij de ziekte van Alzheimer ziekteprogressie kan meten en daarmee een als potentiele nieuwe uitkomstmaat voor interventie-onderzoek kan fungeren (hoofdstuk 4.1,4.2).

Inleiding

De ziekte van Alzheimer is de meest voorkomende oorzaak van dementie. Deze ziekte heeft een grote invloed op het leven van patiënten en hun families, en is een veelvoorkomende oorzaak van overlijden. In de hersenen wordt de ziekte gekenmerkt door ophopingen van de eiwitten amyloïde en tau, wat gepaard gaat met schade aan de hersencellen, en achteruitgang van het denkvermogen. Het proces van hersenkrimp en cognitieve achteruitgang bij de ziekte van Alzheimer duurt jaren, maar is altijd progressief. Uiteindelijk heeft een individu hulp nodig bij activiteiten van het dagelijks leven, en spreken we van dementie. Er is momenteel geen behandeling om de ziekte van Alzheimer te stoppen. Uit eerder onderzoek is wel duidelijk geworden dat kenmerken van de ziekte van Alzheimer lang vóór het begin van dementie in de hersenen aanwezig zijn. Hierdoor is het idee ontstaan dat we personen met biologische aanwijzingen voor de ziekte van Alzheimer moeten behandelen om het ontstaan van dementie uit te stellen, of zelfs voorkomen. Dit concept noemen we in de geneeskunde secundaire preventie.

Een leidende hypothese is dat amyloïde ophopingen in de hersenen een van de eerste kenmerken zijn van de ziekte van Alzheimer (Figuur 1 H1 p.8), vervolgens ontstaan tau ophopingen en daarna hersenkrimp en cognitieve problemen. Het meest innovatieve, en uitdagende, zijn de medicijnonderzoekonderzoeken die

interveniëren in het preklinische stadium van de ziekte van Alzheimer, wanneer er geen objectiveerbare cognitieve problemen, maar al wel biologische kenmerken van de ziekte. De ontwikkeling van dit soort medicijnonderzoeken is echter niet eenvoudig. In het voorstadium van de ziekte van Alzheimer zijn hersenafwijkingen subtiel en de standaard uitkomstmaten om de effectiviteit van Alzheimermedicatie te bepalen onvoldoende gevoelig om verandering over tijd te meten. Onvolledige begrip over het natuurlijke beloop van het voorstadium van Alzheimer-dementie bemoeilijkt het vaststellen van passende selectiecriteria voor deelname aan behandelonderzoek, evenals de ontwikkeling van geschikte uitkomstmaten. Bovendien is een goede werving van onderzoekdeelnemers essentieel, maar het is nog niet duidelijk hoe de deelnemers te vinden en te testen op geschiktheid voor deelname. Hoe secondair preventie-onderzoek bij Alzheimer optimaal vorm te geven en uit te voeren, is dus een actueel onderwerp van wetenschappelijk debat.

Hoofstuk 2 Klinisch beloop van de ziekte van Alzheimer

In **hoofdstuk 2.1** schatten we de duur van de preklinische (amyloïde-positieve normale cognitie), prodromale (amyloïde-positieve MCI) en dementie stadium van de ziekte van Alzheimer in. De schattingen zijn gebaseerd in totaal 3.268 individuen uit meerdere cohorten. We hielden hierbij rekening met leeftijd, geslacht, *APOE*-genotype, een verhoogde liquor tau-concentratie en de populatie (geheugenkliniek of puur onderzoek). De opgetelde duur van de ziektestadia varieerde van 24 jaar op 60-jarige leeftijd tot 15 jaar op 80-jarige leeftijd. Voor een individu in het preklinische stadium, leeftijd 70 jaar, was de geschatte duur opeenvolgend 10 jaar in het preklinische, 4 jaar in het prodromale en daarna 6 jaar in het dementie stadium. Verklaringen voor de kortere totale ziekteduur op hogere leeftijd zijn een langere blootstellingsduur bij presentatie en een lagere veerkracht van de hersenen. We vonden een uitgesproken effect van populatie: zo was op 70-jarige leeftijd de schatting van het preklinische stadium bij puur onderzoek 11 jaar en bij de geheugenkliniek 4 jaar. Mogelijk waren patiënten van de geheugenkliniek al langer in het preklinische Alzheimer-stadium bij presentatie, óf ze hadden een agressievere vorm van de ziekte. De preklinische en prodromale stadia waren één tot enkele jaren korter bij *APOE ε4*-allel dragerschap of een verhoogde liquor tau-concentratie. Dit past bij eerdere bevindingen. Concluderend verbeterden onze schattingen de bestaande kennis over de duur van de ziekte van Alzheimer, en werden deze nauwkeuriger bij rekening houden met leeftijd, geslacht, *APOE-genotype*, liquor tau-concentratie en de onderzochte populatie.

In **hoofdstuk 2.2** onderzochten we individuen die verbeterde na een initiële diagnose van MCI. Tien tot 30 procent van de individuen met MCI vertoont verbetering, en functioneert daarna weer op normaal niveau. Dit lijkt een positieve gebeurtenis. Echter, eerder onderzoek toonde aan dat deze verbeteraars een verhoogd risico op dementie houden. We vermoedden dat dit verhoogde risico te wijten is aan onderliggende ziekte van Alzheimer. We onderzochten welke eigenschappen geassocieerd waren met opnieuw achteruitgang naar MCI of zelfs dementie,

m.b.t. demografie, cognitie, liquor- en beeldvormingsmarkers. We selecteerden de verbeteraars van MCI in twee onafhankelijke onderzoeken, het Alzheimer Disease Neuroimaging Initiative (ADNI) en het Amsterdam Dementia Cohort. De verbeteraars die vervolgens achteruitgang naar MCI of dementie vertoonden waren ouder en hadden vaker een abnormale amyloïde PET-scan en verhoogde liquor tau-concentraties in vergelijking tot de stabiele verbeteraars. Dat betekent dat in deze patiëntengroep, met een niet-typisch klinisch beloop, de Alzheimer-biomarkers een ondersteunende waarde hadden bij het onderscheiden van individuen met een goede prognose, de stabiele MCI-verbeteraars, ten opzichte van hen met een hoger risico om op korte termijn weer achteruitgang te vertonen.

Hoofdstuk 3 Werving van deelnemers voor Alzheimer-preventie onderzoek

Medisch onderzoek loopt vaak vertraging op en wordt regelmatig zelfs nooit afgemaakt, door een gebrek aan onderzoekdeelnemers. In **hoofdstuk 3.1** beschreven we de aanpak bij het European prevention of Alzheimer Dementia (EPAD) project. Voor onderzoek naar preventie van dementie door de ziekte van Alzheimer vindt de werving van deelnemers zowel op bij geheugenpoliklinieken als hierbuiten plaats. De doelgroep heeft alleen lichte of zelfs geen geheugenklachten en -problemen, en bezoekt dus die niet per sé een kliniek. In de afgelopen jaren startten een aantal medicijnonderzoeken met een secundaire preventiestrategie voor individuen met bewezen normale cognitie en amyloïde ophopingen in de hersenen, dan wel genetische risicofactoren, *APOE ε4* genotype en/of *TOMM40*. Die werving ging via advertenties, en gepaard met veel afvallers bij de geschiktheidstesten. Als alternatief voor werving via geheugenpoliklinieken en advertenties zetten we voor het EPAD-project een virtueel register op. Het doel was om de werving verbeteren en het percentage afvallers bij het geschiktheidsonderzoek van het EPAD longitudinale cohortonderzoek (EPAD-cohort) verminderen door voorselectie van individuen uit bestaande studies te faciliteren. Het EPAD-cohort heeft twee doelstellingen; het verbeteren van de kennis over hoe de ziekte van Alzheimer ontstaat, en het vormen van een zogenaamd 'trial-ready cohort voor preventie-onderzoek'. Dit laatste betekent dat deelnemers van de EPAD-cohort kunnen gaan deelnemen aan (nog onbepaalde) interventie-onderzoeken, als ze dat willen en in aanmerking komen.

In **hoofdstuk 3.2** vergeleken we de werving van deelnemers voor het EPAD-cohort in Europa via 4 verschillende routes (geheugenpolikliniek, populatie-cohort, online- en offline-onderzoekdeelnemers). De wervingspercentages waren het hoogst in de geheugenkliniek (59%) en het laagst in het populatie-cohort (3%). Ondanks het verschil in wervingspercentage, waren totale deelnemersaantallen vergelijkbaar tussen de wervingsroutes. Het percentage deelnemers met amyloïde ophopingen was ongeveer 30%. We bekeken ook of de aanwezigheid van risicofactoren voor Alzheimer de werving en de amyloïde status beïnvloedden. We vonden dat individuen die relatief jong waren, hoger opgeleid, een mannelijk geslacht hadden, en/of dementie in de familie hadden vaker bereid en in staat waren om deel te nemen aan het EPAD-

cohort. Onder de deelnemers van het EPAD-cohort, hadden de relatief oudere deelnemers en de dragers van een *APOE ε4*-allel vaker een positieve amyloïde status. De effecten van de voorspellers waren vergelijkbaar tussen de wervingsroutes. De bevindingen laten zien dat deze werving via verschillende routes mogelijk is. De cijfers bieden toekomstige onderzoeken een indicatie van hoeveel mensen te benaderen en testen om een benodigd aantal deelnemers te vinden.

Hoofdstuk 4 Grijze-stofnetwerk van de hersenen, een nieuwe uitkomstmaat voor interventies?

We hebben uitkomstmaten nodig die in predementie-stadia betrouwbaar ziekteprogressie meten om de effecten van nieuwe behandelingen te evalueren. Wij bestudeerden in **hoofdstuk 4.1** of het individuele grijze-stofnetwerk een goede marker vormt van ziekteprogressie bij de ziekte van Alzheimer (zie Panel 1 H4.1 p.116 voor de technische details over deze netwerk-methode). Gebruikmakend van de grijze-stof extracties van de structurele T1 scans, bepaalden we het grijze-stofnetwerk voor elk individu. We berekenden de netwerkmaten, zoals gemiddelde clustering coëfficiënt en het small-world effect, met behulp van grafentheorie. We onderzochten deelnemers van het DIAN-cohort, met leden van families waarin een autosomaal-dominante Alzheimer-mutatie (*APP, PSEN1/2*) voorkomt. Familieleden zonder de mutatie vormen de controlegroep. Bij deze erfelijke vorm van de ziekte van Alzheimer is de leeftijd waarop cognitieve achteruitgang begint erfelijk binnen families. Dat geeft een unieke mogelijkheid om het voorstadium van de ziekte te bestuderen, wanneer we alle deelnemers op een continue tijdslijn van het aantal jaar voor of na verwachte aanvang van dementie in hun familie plaatsen. Het vergelijken van de groep met en zonder de mutatie op elke tijdspunt, geeft inzicht in de dynamische veranderingen van markers over het hele ziekteproces (zie H1 inleiding, figuur 3).

De grijze-stofnetwerken vertoonden gedurende het ziekteproces steeds gelijkenis met willekeurig getekende netwerken. Bij de groep met de mutatie versnellende de achteruitgang in netwerkverstoring vanaf 6 jaar voor diagnose van dementie. Dit was de eerste studie die verergering van netwerkverstoringen over tijd binnen individuen met de ziekte van Alzheimer aantoonde. Het verband tussen netwerkverstoring en ziekteprogressie werd verder ondersteund door de bevinding dat de snelheid van achteruitgang van de grijze-stofnetwerken voorspeld werd door de mate van amyloïde ophopingen. De netwerkverstoring over tijd was ook sterk gecorreleerd met bekende markers van neurodegeneratie, en, belangrijk, met cognitieve achteruitgang. Dit onderzoek biedt daarmee een validatie van grijze-stofnetwerken als marker voor ziekteprogressie bij de ziekte van Alzheimer.

In **hoofdstuk 4.2** onderzochten we welke biologische mechanismen ten grondslag liggen aan verstoringen van het grijze-stofnetwerk, wederom in het DIAN-cohort. We bekeken het verband tussen verstoring van het grijze-stofnetwerk en Alzheimer-gerelateerde liquor biomarkers. De liquor biomarkers reflecteren: amyloïde ($A\beta_{42/40}$) en tau (p-tau) ophopingen, cel-schade (VILIP-1, totaal tau),

synaptische (SNAP-25, neurogranin) en axonale schade (neurofilament light - NfL) en ontstekingsmechanismen (YKL-40, sTREM2). Des te abnormaler de liquor-concentraties van deze markers waren, hoe ernstiger het hersennetwerk verstoord was. De associatie tussen hogere NfL-concentraties en netwerkverstoring was het sterkst. Dit ondersteunt een basale theorie dat axonale integriteit van belang is voor dit hersennetwerk dat berust op covariantie-patronen in de grijze stof. Als laatste bekeken we weer de dynamiek van de biomarkers ten opzichte van de tijd tot familie-specifieke diagnose van dementie. Veranderingen in amyloïde, tau, synaptische en cel-schade-biomarkers traden op in een vroeger stadium dan achteruitgang in de grijze-stofnetwerkmaten. Axonale schade en verhoogde astrocyten-activatie traden op rond hetzelfde moment als de netwerkmaten begonnen af te wijken bij de familieleden met de genetische mutatie. De bevindingen suggereren dat verstoringen van het grijze-stofnetwerk het verlies van axonale connectiviteit bij de ziekte van Alzheimer weerspiegelen, optredend na de eerste schade aan de synapsen en neuronen.

Implicaties voor secondaire-preventie onderzoek

Onze bevinding in hoofdstuk 2 dat het voorstadium van dementie naar schatting 12 tot 17 jaar duurt is relevant voor interventie-onderzoek, omdat het dus lang kan duren voordat een klinisch relevant effect meetbaar is. De resultaten geven ook een verbeterde indicatie van de ziekteduur van alle stadia van de ziekte van Alzheimer, wat van prognostische waarde is. *APOE*-genotype en liquor tau-concentratie waren voorspellend voor snellere progressie in het voorstadium, en hebben mogelijk toepassing om deelnemers verder te stratificeren of verrijken om de power van secondair preventie onderzoek te vergroten. In hoofdstuk 3 zagen we dat de momenteel beschikbare voorspellers voor amyloïde ophopingen niet zo sensitief en specifiek zijn. Voor prescreenings-toepassingen, lijkt de implementatie van de recent ontwikkelde bloedmarkers veelbelovend. Ons onderzoek naar grijze-stofnetwerken liet zien dat deze maat robuust geassocieerd is met ziekteprogressie. Het suggereert dat deze maat verder ontwikkeld zou kunnen worden om als surrogaat uitkomstmaat, mogelijk in combinatie met andere hersenmaten, ziekteprogressie te monitoren.

De snelheid van ziekteprogressie verschilde tussen onderzoekomgevingen/populaties. De voorspellers van ziekteprogressie waren wel vergelijkbaar. Mogelijk kan nog betere biologische en klinische fenotypering dit verschil verkleinen. In de patiëntenpopulatie met preklinische ziekte van Alzheimer lijkt er een zelf-, partner- of zorgverlenerselectie richting 'late-stage' preklinische ziekte van Alzheimer. Vanuit klinisch perspectief zou het optimaal zijn als we dat stadium kunnen herkennen en bevestigen, i.e., vlak voordat er cognitieve problemen ontstaan. Onze bevindingen wijzen erop dat het mogelijk moet zijn om deze individuen te identificeren.

Kanttekeningen

Een probleem bij het bestuderen van een langzaam voortschrijdende ziekte als die ziekte van Alzheimer is dat de 'blootstellingsduur' tussen individuen verschilt bij

aanvang van het onderzoek. De mate en snelheid van voorafgaande hersenschade zijn onbekend, terwijl dit de snelheid van ziekteprogressie beïnvloedt. Stageringsmodellen zijn bedoeld om individuen beter te orderenen op ziekte-ernst. De aanname is dat wanneer de stadiëring binnen preklinische ziekte van Alzheimer preciezer is, de ziekte-gerelateerde afwijkingen er beter uitspringen en de cascade zichtbaar wordt. Het blijven echter modellen, beïnvloed door factoren als de selectiebias, steekproefgrootte, model-specificaties, de exacte definitie van variabelen, en bodem- en plafondeffecten van de markers.

We hebben twee onderzoeken gedaan bij de autosomaal-dominante ziekte van Alzheimer, wat leidt tot vragen over de generaliseerbaarheid van bevindingen naar sporadische ziekte van Alzheimer. Een voordeel van het bestuderen van deze mutatiedragers was de beperkte verouderingseffecten. Dat komt door de relatief jonge leeftijd van ontstaan van cognitieve problemen. Verstoringen van grijze-stofnetwerken waren consistent tussen sporadische en autosomaal-dominantie ziekte van Alzheimer. Met betrekking tot de ontwikkeling van behandelingen is het met name van cruciaal belang om te weten te komen of oorzakelijke cascades tussen deze vormen convergeren. Onze bevindingen suggereren dat convergentie eerder optreedt in het ziekteproces dan de netwerkverstoringen.

Toekomstperspectieven en conclusie

Ziekte-modellen

Nu datasets in de afgelopen jaren snel groter worden en individuen met biomarker-metingen steeds langer gevolgd zijn over de tijd, starten we met het toepassen geavanceerdere methoden om de ziekteprogressie in het voorstadium van de ziekte van Alzheimer beter te begrijpen. Met een precieze individuele prognose zouden we in de toekomst precies op tijd; voor het ontstaan van cognitieve problemen, maar zo laat mogelijk een behandeling kunnen starten.

Deelnemersregisters

De werving van deelnemers via lopende onderzoeken bij het EPAD-project werkte goed om bij de start van het project vlot van slag te gaan, en het aantal deelnemers dat niet door het geschiktheidsonderzoek kwam was relatief laag. Echter voor een duurzame oplossing voor werving van deelnemers is een continue investering nodig. Evenals onderzoekers wereldwijd, zijn wij daarom met Hersenonderzoek.nl gestart, onder leiding van Marissa Zwan en Niels Prins. Dit is een online platform waarop alle Nederlandse hersenwetenschappers deelnemers kunnen werven. We hebben geconstateerd dat de opzet en het onderhoud van een online platform expertise vereisen over zowel de ziekte van Alzheimer, als online werving, als wetenschapscommunicatie, als technologische aspecten. Een generiek register gaat dus gepaard met aanzienlijke kosten, maar vermindert de wervingsinspanningen in andere projecten en verbetert waarschijnlijk zelfs het percentage studies met een succesvolle voltooiing van de inschrijving. Toch zal

voorlopig een uitdaging blijven om de waarde van deze registers aan te tonen, omdat de wervingsinspanningen en -tijd vaak worden onderschat, en regelmatig niet eens in project-begrotingen voorkomen.

'Run-in data' voor selectie en evaluatie van resultaat bij interventie-onderzoek
Voor de evaluatie van interventie-onderzoek is aangetoond dat een aanloopperiode ('run-in' = waarbij deelnemers al worden gevolgd, maar nog niet behandeld) de power van de studie kan verbeteren ten opzichte van het negeren van het natuurlijke ziektebeloop voor randomisatie voor de interventie. Een aanloopperiode is al geïmplementeerd in DIAN-TU-medicijnonderzoeken en het EPAD-project, wat ervoor dat het aantal benodigde deelnemers per project lager is dan bij een traditioneel design. Toekomstige medicijnonderzoeken kunnen het onderzoeksdesign met een aanloopperiode verfijnen en optimaliseren, en mogelijk ook implementeren voor selectie van de deelnemers voor een onderzoek.

Motivatie en betrokkenheid van onderzoekdeelnemers, waarom en hoe?
Zonder deelnemers is onderzoek niet mogelijk. Deelname aan onderzoek ter preventie van dementie, en zeker medicijnonderzoek, past niet bij ieders persoonlijke omstandigheden, levensstijl en persoonlijkheid. Motivaties omvatten aspecten van: dementie in de persoonlijk sfeer, altruïsme, bijdragen voor de volgende generatie, passie voor wetenschap, zorgen over cognitie, nieuwsgierigheid naar het lichaam, zingevende activiteit, uitzicht op frequente controles of hoop op persoonlijk voordeel. Interactie met de preventie-onderzoekdeelnemers kan ons leren welke praktische aspecten motiverend en belemmerend werken. Dit biedt weer richting voor de verlaging van de lasten voor de deelnemers, wat de werving (en retentie) kan verbeteren.

Conclusie
In dit proefschrift hebben we het beloop van de ziekte van Alzheimer onderzocht met verschillende methoden en figuur 1 in H.5 plaatst de bevindingen in de context van pathologische cascade van deze ziekte. De opgedane kennis is van belang voor begrip over het ziektebeloop, het structureren van toekomstige interventie-onderzoeken in verschillende ziektestadia, en de implementatie van behandelingen wanneer deze beschikbaar komen. Momenteel is het meeste secundaire preventie-onderzoek gericht op amyloïde ophopingen, ook de voornaamste focus in onze studies. Relatief nieuw is dat er middelen voor meer diverse aangrijpingspunten ver zijn in de ontwikkeling richting klinisch onderzoek, die onder meer anti-tau-middelen bevatten. Daarom zullen duurzame deelnemersregisters met diverse samenstelling en betere bloed biomarkers nodig zijn om flexibiliteit te bieden voor veranderende selectiecriteria. Met onze moderne tools, onvermoeibare inspanningen van onderzoekers en deelnemers, en geïnspireerd door recente behandelingssuccessen bij andere neurologische aandoeningen, zou er zo maar een doorbraak aan kunnen komen.

Dankwoord

Allereerst wil ik de onderzoekdeelnemers en hun naasten bedanken voor hun deelname aan de onderzoeken, van DIAN, ADNI, AIBL, Amsterdam dementie cohort, H70, DESCRIPA en ICTUS en in het bijzonder de deelnemers van EPAD. U maakt de onderzoeken uit dit proefschrift mogelijk. Het zijn uiteindelijk de deelnemers en patiënten die mij inspireren, en zeker ook uitdagen, om de meest complexe, relevante problemen te onderzoeken.

Verder gaat mijn dank uit naar mijn collega's die overal ter wereld, van Europa tot Australië, Amerika tot Japan, en in het Alzheimercentrum Amsterdam, gegevens hebben verzameld en beschikbaar gemaakt om de vraagstellingen van dit proefschrift te beantwoorden.

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we nog blijven! PJ's angels – mijn meest waardevolle ongestuurde bijeenkomst van de week. Samen op tournee naar Edinburgh, Chicago, LA, St. Louis, Providence on Rhode Island, met de AC-campertrippers, kanoën, dansen, of gewoon kletsen over alles wat ons bezighoudt, Revues, Concertgebouw, Betty's promovendi-uitjes, de AAFW, SAIL, Vrijdagmiddelborrels, Pizzeria's, Brusselse avonturen, CTAD Barcelona, en schrijf-retraite in Mallorca. Bij elkaar echt heel bijzonder, en ik heb het als een enorme steun en stimulans ervaren om tijdens mijn PhD-traject zoveel leuke collega's om me heen te hebben. Ik ben dankbaar voor de waardevolle vriendschappen en werkrelaties die we hebben opgebouwd en versterkt.

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Nederlandstalige blogs voor algemene publiek

Tijdens mijn PhD-periode heeft Astrid Hooghiemstra me aangespoord om blogs over mijn onderzoek te schrijven. Samen vormen de blogs een natuurlijke samenvatting van dit boek, vandaar ik dat ik het passend vond ze hieronder nogmaals te publiceren.

De duur van de ziekte van Alzheimer ingeschat (H2.1)

Door: Lisa Vermunt, Sietske Sikkes, Pieter Jelle Visser

De ziekte van Alzheimer komt veel voor, maar niemand weet hoe lang de ziekte van Alzheimer duurt en hoe dat per persoon verschilt. Sinds enige jaren kunnen we de biologische kenmerken van de ziekte van Alzheimer vaststellen met biomarkers. Biomarkers zijn lichaamseigen stofjes die iets zeggen over de processen die spelen in het lichaam en die we kunnen meten met speciale apparatuur. In dit onderzoek, gepubliceerd in *Alzheimer's & Dementia*, knoopten we internationale gegevens aan elkaar van mensen die deze allereerste biologische afwijkingen hadden. Met een wiskundige schatting berekenden we de duur vanaf die allereerste afwijkingen, het voorstadium, tot aan het eindstadium van de ziekte van Alzheimer.

Het belangrijkste resultaat was dat het voorstadium van de ziekte van Alzheimer wel meer dan 17 jaar kan duren. Voor oudere mensen was de duur korter dan voor jongere mensen. Ook voor mensen met een genetisch risico, tau-eiwitstapeling, of als mensen hulp hadden gezocht voor geheugenklachten was de duur korter. Daarnaast vonden we dat de gemiddelde overlevingsduur van mensen met dementie gemiddeld 8 jaar was, en dat deze langer was voor vrouwen dan voor mannen.

Voor deze methode waren een hoge computerkracht en veel gegevens nodig. Dit laatste was mogelijk door een grote internationale samenwerking. We mochten gegevens gebruiken uit de Verenigde Staten, Australië en Europa. Hierdoor hadden we een grote groep om een betrouwbaardere schatting over de ziekteduur te maken. Met de resultaten kunnen artsen hun patiënten in verschillende stadia beter informeren over het ziektebeloop. Mocht er in de toekomst een behandeling komen voor de ziekte van Alzheimer, dan kunnen deze resultaten helpen om het verwachte effect en het startmoment te bepalen.

Alzheimer biomarkers helpen te voorspellen of verbetering van cognitie blijvend is. (H2.2)

Door: Lisa Vermunt, Betty Tijms

Mensen met milde cognitieve stoornissen hebben een verhoogde kans om dementie te ontwikkelen. Het opvallende is dat er bij een kwart van deze mensen soms een verbetering van de klachten optreedt. Hoewel verbetering een positieve verandering

is, liet eerder onderzoek zien dat deze 'verbeteraars' toch nog steeds een verhoogd risico hebben om dementie te ontwikkelen. Die bevinding geeft onzekerheid bij artsen en patiënten. Mensen met een verhoogd risico zou je immers willen uitnodigen voor herhaalbezoeken, terwijl anderen zonder verhoogd risico juist niet terug hoeven te komen. Maar hoe maak je dat onderscheid?

Onderzoek en resultaten

Om daarachter te komen, bestudeerden wij gegevens van deze 'verbeteraars'. In ons onderzoek vergeleken we twee groepen mensen die een verbetering van geheugenklachten lieten zien. De eerste groep waren 'stabiele' verbeteraars en de tweede groep bestond uit mensen bij wie de verbetering tijdelijk was. We onderzochten de uitslagen van geheugen- en aandachts-testen, de hersenscans en we keken naar aanwijzingen voor de ziekte van Alzheimer in het hersenvocht, namelijk klontering van de eiwitten amyloïd-bèta en tau.

We kwamen tot de conclusie dat alzheimer biomarkers na initiële verbetering van geheugenproblemen kunnen bijdragen aan de voorspelling wie langdurig gezond blijft en bij wie de verbetering van klachten tijdelijk is. De groep die verbeterde en stabiel bleef was relatief jonger en de hersenfoto en de alzheimer biomarkers waren vaker normaal. We denken dat de verbeteraars mogelijk slecht hadden gescoord op de geheugentesten doordat ze wellicht nerveus waren voor de testen, en een enkeling door depressieve klachten. In de groep die slechts tijdelijk verbeterde zaten meer oudere en hoogopgeleide mensen, die op de testen net te laag hadden gescoord. Deze groep mensen, bij wie de verbetering tijdelijk was, hadden juist wel aanwijzingen voor hersenschade op de scan en voor de ziekte van Alzheimer in het hersenvocht. We weten nog niet goed waarom de mensen tijdelijk verbeterden. Uit dit onderzoek blijkt opnieuw dat alzheimer bij iedereen anders verloopt.

Belang van het onderzoek

De resultaten van het onderzoek geven handvatten aan artsen om hun patiënten een nauwkeurigere prognose te geven. Daarmee kunnen ze de patiënten beter informeren en hun begeleiding aanpassen aan de patiënt.

De methode van werving voor dementie-preventie onderzoek is nog niet uitgekristalliseerd (H3)

Door: Lisa Vermunt, Marissa Zwan

Klinische onderzoeken worden zeer regelmatig niet afgerond doordat er onvoldoende geschikte deelnemers worden gevonden. Dat is natuurlijk zonde van het geld, maar zeker ook van de moeite van de deelnemers die wel meededen. Het is dus belangrijk goede wervingsmethoden te ontwikkelen en deze te blijven verbeteren. Biomarker-onderzoek naar het ontstaan en voorkómen van dementie door de ziekte van Alzheimer is relatief nieuw. Een eerste stap is om reeds bestaande wervingsmethoden met elkaar te vergelijken. Dat deden we bij het Europese 'EPAD-cohort' onderzoek.

Uitvoering en resultaten

Verschillende wervingsroutes werden vergeleken: een online-onderzoekregister (Hersenonderzoek.nl) in Nederland, een geheugenkliniek in Frankrijk, een offline onderzoekregister in Spanje en een gezondheidsonderzoek in Schotland. Via elke route waren voldoende deelnemers voor onderzoek te vinden, alleen de slagingspercentages verschilden. Op basis van onze bevindingen kunnen we nu beter inschatten hoeveel méér aanmeldingen in een register nodig zijn, ten opzichte van patiënten in de polikliniek om hetzelfde aantal onderzoekdeelnemers te vinden. De beste methode hangt af van de lokale expertise en specifieke doelstellingen.

We vergeleken ook of kenmerken de daadwerkelijke deelnemers van het EPAD-cohort met degenen die niet wilden of konden deelnemen. De uiteindelijke deelnemers waren relatief jonger, vaker hoger opgeleid, vaker man en dementie kwam vaker voor in de familie.

Interventie-onderzoeken richten zich tegenwoordig vaak op mensen met amyloïde (Alzheimereiwit) ophopingen in de hersenen. We onderzochten daarom ook welke eigenschappen daarmee samenhingen. Deze bleken juist verband te houden met een oudere leeftijd, en met een bepaalde genetische variant. De werving voor klinische onderzoeken naar het ontstaan en voorkómen van dementie door de ziekte van Alzheimer zou dus kunnen verbeteren als meer relatief oudere mensen zouden deelnemen, omdat zij een hogere kans hebben op het hebben van amyloïde ophopingen.

We hebben in dit onderzoek niet precies bijgehouden waarom mensen niet konden of wilden deelnemen. Wel zagen we dat medische problemen van henzelf of hun partner vaak een rol speelden. Een mogelijke oplossing zou zijn om deelname aan onderzoek minder belastend te maken, bijvoorbeeld door het onderzoek gedeeltelijk vanuit huis uit te voeren.

EPAD

Dit onderzoek was mogelijk door samenwerking met collega's in heel Europa in het kader van EPAD. Het project loopt nog steeds en wordt gesteund door de Europese Unie in een publiek-private samenwerking met bedrijven. Het EPAD-cohort heeft nu bijna 2.000 deelnemers en dankzij hun medewerking kunnen de beschikbare onderzoeksgegevens ook worden gebruikt voor onderzoek door onze (inter)nationale collega's naar het ontstaan en voorkómen van dementie.

Naar het buitenland met Alzheimer Nederlandbeurs (H4 - 1)

Door: Astrid Hooghiemstra, Lisa Vermunt

Eind vorig jaar ontvingen 4 promovendi een beurs van Alzheimer Nederland die het mogelijk maakt om enkele maanden te verblijven en mee te lopen bij een buitenlandse onderzoeksgroep. De kennis en ervaring die de jonge onderzoekers opdoen tijdens deze periode vormt een waardevolle aanvulling voor hun promotieonderzoek. Wat ze precies gaan doen en hopen te leren in het buitenland? Lees het hieronder.

Met de Alzheimer Nederland subsidie ga ik naar Washington University in St. Louis, USA. Binnenkort sluit ik me tijdelijk aan bij het onderzoeksteam van het DIAN-onderzoek van Washington University. Het DIAN-onderzoek is uniek in de wereld. In het DIAN-onderzoek worden al jaren families gevolgd met het mensen met een afwijking in een van de Alzheimer-genen. Veel familieleden met een afwijking in het Alzheimer-gen hebben nog geen klachten, maar doen toch mee. Daardoor geven ze wetenschappers de kans om het voorstadium van de ziekte van Alzheimer beter te leren begrijpen. Het is een grote kans voor mij om veel te leren, want het DIAN-onderzoeksteam heeft veel expertise zowel inhoudelijk, als in de samenwerking met hun deelnemers. De groep waar ik bij ga werken verdiept zich in hersenscans en staat onder leiding van dr. Tammie Benzinger. We gaan de hersenscans gebruiken om te kijken hoe de samenwerking tussen hersengebieden verandert in het voorstadium van de ziekte van Alzheimer. Een verandering in de samenwerking tussen hersengebieden noemen we ook wel een 'netwerkverstoring'. We denken dat dit een van de eerste signalen is van de ziekte van Alzheimer. We willen netwerkverstoring bij Alzheimer beter begrijpen, zodat we beter kunnen meten welk vooruitzicht iemand heeft. Bent u nieuwsgierig waarom het meten van 'netwerkverstoring' ook belangrijk is voor medicijnonderzoeken? Houd dan mijn blog in de gaten dat ik zal bijhouden tijdens mijn bezoek aan Washington University.

'Meet me in St. Louis' (H4 - 2)

Door: Lisa Vermunt, redacteur: Astrid Hooghiemstra

Vorig jaar ontving ik een bijdrage van Alzheimer Nederland om een periode in het buitenland ervaring op te doen. Ik wilde graag naar Washington University (St. Louis, Missouri, VS) vanwege de vooruitstrevende bijdragen aan het dementieonderzoek. St. Louis is bij ons Nederlanders minder bekend als stad. De stad ligt in de 'mid-West' van de Verenigde Staten. Het heeft de hoofdrol in de muziekfilm 'Meet me in St. Louis' uit 1944. In deze film zingt Judy Garland de 'Trolley song'. De film speelt zich af in de hoogtijdagen van de stad, in 1903. Dat was het jaar voorafgaand aan de wereldtentoonstelling en de Olympische spelen alhier. Eens was St. Louis de 4e stad van de Verenigde Staten. Nu is van die grootsheid nog maar weinig te zien, met als uitzondering Washington University.

Bij Washington University is de bravoure duidelijk zichtbaar. Al in de jaren '70 speelde de onderzoekers van Washington University een belangrijke rol bij de ontwikkeling van de PET-scan (Positron Emission Tomography). Het gebouw waar ik werk, herinnert me dagelijks aan deze geschiedenis. Er staan meerdere van deze oude PET-scanners in het gebouw tentoongesteld. Ik heb er twee op de foto gezet.

Wat is een PET-scan eigenlijk? De PET-scan maakt gebruik van radioactieve stofjes om processen in de hersenen zichtbaar maken. De scans zien er tegenwoordig anders uit, maar werken op dezelfde manier. Op mijn foto's zijn computerchips te zien. Dit zijn gevoelige ontvangers van de radioactieve straling. Ze zitten rondom het gat waar iemand zijn hoofd in legt. Doordat ze helemaal rondom zijn geplaatst,

verschilt het opgevangen signaal tussen de ontvangers. De signalen gaan naar een computer, die ons vertelt uit welk deel van de hersenen elk signaal komt. Zo konden onderzoekers voor het eerst de energiehuishouding van de hersenen bekijken. Dit bleek verslechterd bij Alzheimerpatiënten in vergelijking met mensen zonder de ziekte. Het onderzoek naar nieuwe stofjes om herseneiwitten of hersenprocessen zichtbaar te maken met de PET-scan, is in de afgelopen jaren enorm ontwikkeld. We denken dat dit belangrijk is om de ziekte van Alzheimer beter te herkennen en begrijpen.

Op mijn afdeling werkt professor Raichle, een van de uitvinders van de eerste PET-scan. Hij is inmiddels 81 jaar, maar nog steeds volop aan het werk. Ik heb zijn PhD-studenten gevraagd naar zijn geheim. Wat zij zeiden? 'Hij vindt het werk te leuk en heeft nog veel te veel om uit te zoeken.' Daar sluit ik me volledig bij aan. Ik werk hard, maar heb nog meer te doen om mijn project hier te voltooien. Ons project over netwerkverstoring bij de ziekte van Alzheimer zal ik mijn volgende blog verder uitleggen. De stad St. Louis heeft ook veel te doen om de bravoure van weleer terug te vinden. De gemeente en vrijwilligers werken aan een veilige stad en meer gelijke kansen. Wellicht komen dan de Olympische zomerspelen een keer terug in St. Louis.

Netwerkverstoringen in de hersenen belangrijk voor medicijnonderzoeken? (H4 - 3)

Door: Lisa Vermunt, redacteur: Betty Tijms

Bij Washington University in St Louis bestudeer ik 'netwerkverstoring' op de hersenscan van het DIAN-onderzoek (Dominantly Inherited Alzheimer Network). In dit onderzoek doen families mee waar de erfelijke variant van de ziekte van Alzheimer voorkomt. Bij deze zeldzame vorm van Alzheimer begint dementie op jonge leeftijd, vaak al tussen het 35e en 55e levensjaar. Alle familieleden kunnen meedoen aan DIAN. Degenen zonder afwijking in het Alzheimer-gen vormen de 'controlegroep'. Door de controlegroep met de dragers van genetische afwijking te vergelijken, kunnen we erachter komen welke veranderingen te maken hebben met de ziekte van Alzheimer en welke met normaal ouder worden.

Binnen het DIAN-onderzoek nemen sommige mensen ook deel aan medicijnonderzoek. DIAN-onderzoekers en farmaceuten bestuderen of nieuwe medicijnen de ziekte kunnen remmen. Alleen mensen met de genetische afwijking kunnen meedoen aan deze medicijnonderzoeken. Sommige van de deelnemers aan het medicijnonderzoek hebben nog geen klachten. Bij hen is het doel om het ontstaan van klachten uit te stellen.

Maar, als iemand nog geen klachten heeft, hoe kun je dan bepalen of een medicijn werkt en klachten remt? Metingen die als doel hebben om de werkzaamheid van een nieuw medicijn te beoordelen, noemen we 'uitkomstmaten'. Hoe preciezer de uitkomstmaat, sneller bekend is of een middel werkzaam is. Om nieuwe uitkomstmaten te maken bestuderen we wat er in de hersenen verandert bij de mensen met de afwijking in het Alzheimer-gen, die in het voorstadium zijn van de ziekte van Alzheimer.

Ons vermoeden is dat 'netwerkverstoring' op de hersenscan een goede uitkomstmaat kan zijn, omdat we uit eerder onderzoek weten dat netwerkverstoring optreedt voordat er klachten ontstaan. We weten alleen nog niet wanneer het optreedt en hoe netwerkverstoring samenhangt met andere kenmerken van Alzheimer.

Daarom, onderzoeken we nu in het DIAN-onderzoek wanneer in de ziekte netwerkverstoringen beginnen. In onze eerste resultaten zien we dat ongeveer 5 jaar voordat iemand klachten krijgt, netwerkverstoring optreedt. Verder blijkt dat netwerkverstoring samenhangt met geheugenproblemen en ophoping van het 'amyloïde eiwit'. Er is ook een lichte mate van netwerkverstoring bij normale veroudering. In een volgende stap kijken we hoe onze metingen veranderen bij mensen die meerdere hersenscans hebben ondergaan.

Net als ik, werken meer onderzoekers van het DIAN-team aan nieuwe uitkomstmaten om medicijnonderzoeken te verbeteren. De ideale uitkomstmaat is naast zeer precies, ook zo min mogelijk belastend voor de deelnemers van medicijnonderzoeken. De ambitieuze hoofdonderzoeker van DIAN, dr. Bateman, probeert een bloedtest te ontwikkelen die het beloop van de ziekte van Alzheimer kan meten. Dat blijkt niet zo makkelijk, maar gelukkig deinzen ze hier niet terug voor een flinke uitdaging. Dat geldt ook voor alle deelnemers van het DIAN-onderzoek, die het onderzoek mogelijk maken, en vaak het vliegtuig nemen om deel te kunnen nemen.

Als je ver wilt komen, ga dan samen. (H4 - 4)

Door: Lisa Vermunt, redacteur: Melanie Bremer

Dat was een spannende week. Na een aantal maanden onderzoek te hebben gedaan aan Washington University, mocht ik mijn resultaten presenteren. Tijdens het voorbereiden van de presentatie keek ik terug op mijn bezoek.

Aan Washington University heb ik samen met het team aldaar me ingezet om preciezere uitkomstmaten voor medicijnonderzoek te ontwikkelen (meer info vind je in mijn vorige blog). Om dit te doen hebben wij 'netwerkverstoringen' bestudeerd op de hersenscans van het DIAN-onderzoek (Dominantly Inherited Alzheimer Network). Hiervoor gebruikten we een zogenaamde 'supercomputer' en heel veel verschillende gegevens (data). Dit vergt nogal wat van de infrastructuur, want al die data moet opgeslagen en onderhouden worden. Toen ik aankwam viel me meteen op dat de excellente reputatie van Washington University volledig terecht is. Iedereen, van de professoren tot het ICT-team, is erg toegankelijk. Dat is ook hard nodig, want om dit soort toponderzoek uit te voeren moet iedereen nauw samenwerken. Gelukkig had ik het werken met de 'supercomputer' snel onder de knie. Mede dankzij een toegewijde programmeur, bij wie ik altijd kon binnenlopen met vragen. Dankzij het hele team had ik binnen de paar maanden dat ik op bezoek was alle gegevens verzameld voor mijn presentatie.

Presenteren voor een onderzoeksgroep kan best spannend zijn. Wetenschappers staan erom bekend dat zij kritische vragen stellen. Voor een buitenstaander kan dat ongemakkelijk overkomen. De bedoelingen zijn echter goed, door kritische vragen te

stellen help je elkaar verder. Die middag liep ik tijdens mijn presentatie vast in een paar technische details. Een aantal Wiskundige onderzoekers die aanwezig waren stelden pittige vragen. Gelukkig kon ik het later opzoeken en alsnog toelichten. Dat was voor ons allemaal nuttig. Het deed me denken aan een Afrikaans spreekwoord dat een Washington University professor gebruikte. Vrij vertaald luidt het: 'Als je snel wilt gaan, ga dan alleen. Als je ver wilt komen, ga dan samen.'

De trouwe volgers van mijn blogs willen vast weten welke resultaten ik heb gepresenteerd. Kan onze maat van netwerkverstoring gebruikt worden als uitkomstmaat voor medicijnonderzoeken? Het antwoord is gelukkig positief: Jazeker, onze resultaten ondersteunen dit. Maar, de hersenscan kan het ook niet alleen, het is niet specifiek genoeg als uitkomstmaat. De netwerkmaat van de hersenscan kan het beste gecombineerd worden met geheugentesten en andere hersenscans om medicijnonderzoeken sneller te laten verlopen en eerder tot een behandeling te komen.

About the author

Lisa Vermunt was born on the 5th of September 1986 in Tilburg, the Netherlands. During childhood, the possibility of her parents reading her Bayesian and Latent Class Model fairy tales cannot be excluded, which may have affected her scientific interest during early adulthood. After completing her pre-university education *cum laude*, her fascination for the brain led her to Psychobiology at the University of Amsterdam, and she graduated *cum laude*. Highlights included a semester at McGill University in Canada and an internship on early life stress in rats. She studied Medicine at the same university. After graduating as MD in 2014, she worked as neurology ward doctor at Flevoziekenhuis in Almere. Her PhD position under guidance of prof.dr. P.J. Visser, dr. B.M. Tijms, and prof.dr. P. Scheltens in the Alzheimer Center Amsterdam, on the European Prevention of Alzheimer Dementia (IMI-EPAD) project fitted her long-time interest in clinical trials for brain diseases. During the first years of her PhD, she was responsible for the Amsterdam site of the EPAD Registry and Cohort. Her scientific work initially focused on recruitment science, and later on disease modeling in predementia stages, with a special interest for autosomal dominantly inherited Alzheimer disease. In 2018, she visited Washington University (St. Louis) to perform research on the Dominantly Inherited Alzheimer Network (DIAN) cohort. So far, Lisa's work led to two oral presentations at the Alzheimer's Association International Conference, four publications, coverage on Alzforum, and 140 Twitter followers (ratio 1.19).

When Lisa is not following online courses on statistical modelling (completely voluntary) or keeping up with all the literature on Alzheimer's (and sharing her novel theories with colleagues), Lisa spends her spare time on a range of extracurricular activities, from singing in a choir, to setting up randomized controlled cola trials, and driving around a 24-foot camper on the Canadian highways with academic peers (including Dr. Jutten who kindly contributed to the writing of this biography).

Lisa now works as postdoctoral researcher at the Teunissen clinical neurochemistry lab at Amsterdam UMC where she continues her research on the secondary prevention of Alzheimer disease. She focusses on disentangling Alzheimer disease progression by applying diverse statistical approaches to clinical, fluid biomarker and proteomic measurements. Thereby, she is still affiliated to the Alzheimer Center Amsterdam for clinical work and the EPAD project.