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# SUBJECTIVE COGNITIVE DECLINE

Predictive value of biomarkers in the
context of preclinical Alzheimer's Disease



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VRIJE UNIVERSITEIT

# SUBJECTIVE COGNITIVE DECLINE

Predictive value of biomarkers in the
context of preclinical Alzheimer's Disease

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor
aan de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof. dr. V. Subramaniam,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Geneeskunde
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De Boelelaan 1105.

door
Rosalinde Elisabeth Rafaella Slot
geboren te 's-Gravenhage

# CONTENTS

En la forest de Longue Attente
chevauchant par divers sentiers
m'en viys, ceste année présente
où voyage de Desiriers.
Devant sont aller mes fourriers
pours appareiller mon logis
en la Cité de Destinée.
et pour mon Coeur et moy ont pris
l'ostellerie de Pensée.

Charles van Orléans

# CHAPTER 1
General introduction, aims and outline

Chapter 1

**INTRODUCTION**

**Dementia due to Alzheimer's disease**
Dementia is a major global challenge, with an increasing prevalence as life expectancy increases [1,2]. Dementia places an major burden on society and is suggested to affect 65 million people worldwide by 2030 [3]. Alzheimer's disease (AD) is the most common cause of dementia, before vascular cognitive impairment, frontotemporal dementia and dementia with Lewy bodies [2]. The pathogenesis of AD unfolds gradually, and the first pathophysiological changes occur already decades before a diagnosis of dementia [4,5]. It is hypothesized that abnormal amyloid-beta (Abeta) deposition in the brain is one of the first events in the pathogenesis of AD, already starting twenty to thirty years before the onset of dementia due to AD (figure 1) [6–8]. The amyloid hypothesis states that abnormal Abeta deposition is followed by the formation of neurofibrillary tangles, eventually leading to synapse loss and neuronal injury causing cognitive deficits [4,9–11]. The amyloid hypothesis has been discussed extensively [4,5,10–12]. Recent insights suggest roles for both amyloid-dependent and amyloid-independent pathways contributing to the pathogenesis of AD, influencing each other's effects [13,14]. Amyloid-independent processes associated with AD include the contribution of vascular pathology, alterations in lipid metabolism and immunological processes [4,15,16]. It is, however, challenging to combine and weigh these possible contributing factors to the pathogenesis of AD in research and clinical context. Recent efforts by the National Institute of Aging and Alzheimer's Association workgroup have led to a new research framework for Alzheimer disease combining information from different biomarker categories (amyloid, tau and neurodegeneration) [17,18]. Research participants are then classified based on the presence of amyloid and tau biomarkers, rather than the presence of clinical symptoms, emphasizing the need to evaluate AD from a biological perspective.

Up to now, no effective treatment options are available, possibly because trials were executed in patients already in the dementia stage of AD, when neurodegenerative changes may have caused considerable irreversible damage [19]. The lack of treatment options once the dementia stage is reached, together with the evidence of amyloid pathology years before a diagnosis of dementia, have led to an important shift in research focus from dementia to pre-dementia stages of AD.

**Pre-dementia stages of AD**
Mild Cognitive Impairment (MCI) refers to a pre-dementia stage when cognitive deficits are already present, but without detectable interference in daily functioning [20,21]. Individuals with MCI have an increased risk of progression to dementia due to AD, and prevalence of amyloid pathology is higher in MCI compared to the normal population [22,23]. The prevalence of amyloid pathology in non-demented elderly increases with age to around 70% in MCI patients of 90 years and older [7]. Looking even earlier in the disease process, research increasingly focuses on the so-called preclinical stage of AD. Preclinical

AD is defined as an asymptomatic stage of AD, in which AD biomarkers are aberrant, but clinical symptoms of objective cognitive decline are not present [8,24]. Some individuals with preclinical AD may be aware of some decline in functioning, even when this cannot be objectified by neuropsychological testing.
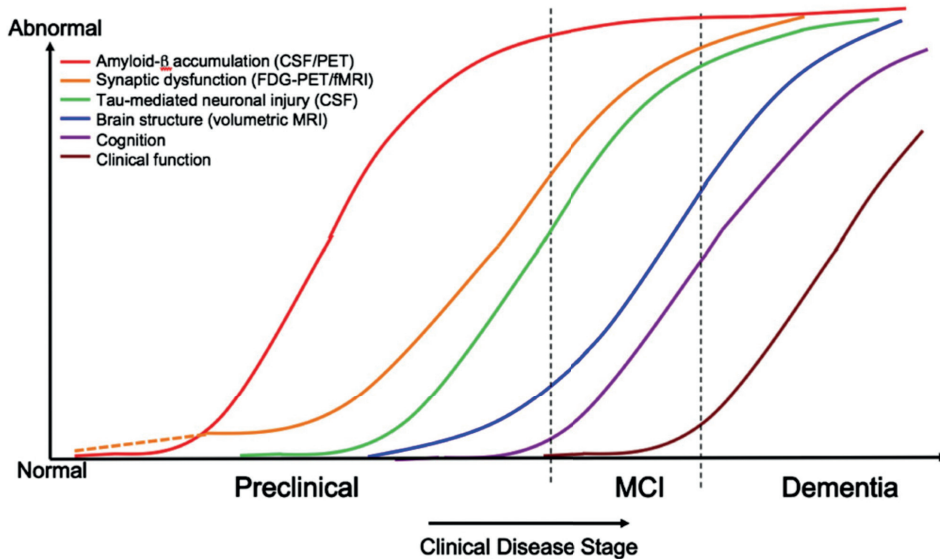


Figure 1. Hypothetical model of dynamic biomarkers of the AD continuum (Sperling et al., 2011)[8]

**Subjective cognitive decline**

Subjective cognitive decline (SCD) refers to the experience of cognitive decline, without formal deficits on neuropsychological testing, nor any other neurological or psychiatric diagnosis explaining cognitive complaints [25]. The concept of subjective cognitive decline was first introduced in 1982 by Reisberg and others, describing 7 stages of AD, in which subjective complaints were designated as stage 2 [26]. Of note, these stages were recently re-introduced in a modified fashion in the ATN model describing the continuum of AD incorporating biomarkers [27]. SCD was defined as memory complaints in individuals with normal cognition, and suggested to be a pre-dementia stage of AD. In the following years Jonker and others investigated the risk of dementia in cognitively normal individuals in with memory complaints in the Netherlands, and observed an increased risk of dementia in SCD [28,29]. About thirty years later the SCD concept gained interest in the context of attempts at understanding the pre-dementia stages of Alzheimer's disease. SCD was referred to with various titles, such as subjective cognitive impairment, and subjective memory complaints [30]. To harmonize the concept of SCD, in 2014 a group of SCD researchers (the SCD-Initiative (SCD-I)) published a conceptual framework on SCD, aimed at facilitating research of and clinical approach to SCD [25]. From then on, the label subjective cognitive decline is most frequently used,

also in this thesis. The subjective experience of cognitive decline has been suggested to be one of the first symptoms of AD, and individuals with SCD have an increased risk of progression to MCI or dementia, especially when complaints are reported by both patient and informant [31–36].

It remains difficult to clinically identify preclinical AD in cognitively healthy individuals who subjectively experience cognitive decline. To increase the likelihood of preclinical AD in individuals with SCD, the SCD-I working group has proposed the SCD plus criteria, published in the conceptual framework on SCD by the international Working Group (SCD-I) [22]. These criteria include biomarkers such as APOE e4 carriership, but also patient specific features such as predominant self-perceived memory decline and feeling of worse memory performance than others of the same age. The SCD plus criteria have been proposed to facilitate harmonizing SCD research, but they have not yet been prospectively validated.

Even though individuals with SCD on average have an increased risk of AD, most people with SCD do not harbor Alzheimer pathology. Alternative potential explanations for the experience of memory problems in cognitively healthy individuals include subthreshold symptoms of affective disorders, personality features, lifestyle factors or systemic illnesses [37–39]. To evaluate the contribution of different factors related to SCD and the natural course of their signs and symptoms in relation to biomarkers, we have set up the memory clinic based Subjective Cognitive Impairment Cohort (SCIENCe). In this ongoing cohort study we investigate individuals with SCD, without major psychiatric or neurological disorders. A large part of the research in this thesis involves participants with SCD of the SCIENCe project and Amsterdam Dementia Cohort. One of the main goals of this thesis was to discriminate those SCD individuals at risk of AD from those with a low risk of AD.
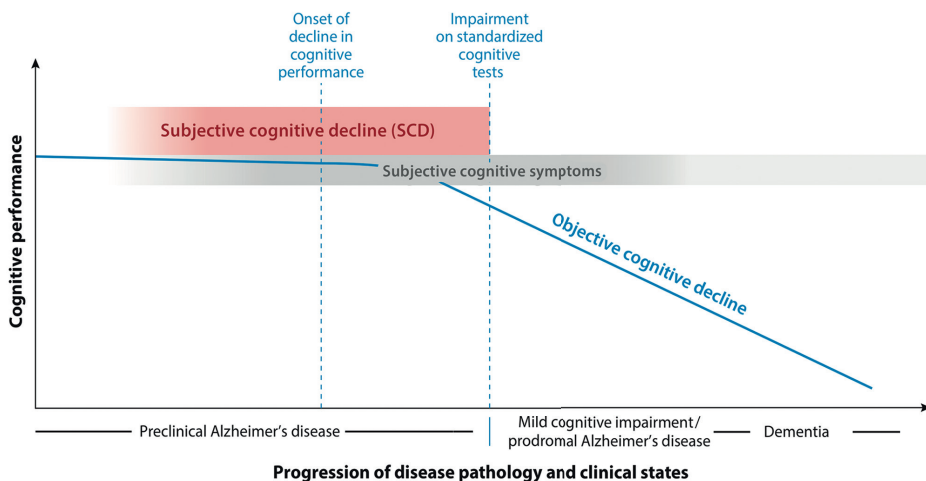


Figure 2. Subjective Cognitive Decline in the AD continuum (Rabin et al., 2017)[30]

While on a group level CSF and imaging markers predict an increased risk of clinical progression, it is difficult for the clinician to weigh and translate findings to the individual with SCD. We aimed to combine biomarker information to predict risk estimates on an individual level. The sequence of neurodegenerative changes eventually leading to AD may vary amongst individuals, and where to place SCD in these pathological sequences remains to be elucidated (figure 2).

**Biomarkers in SCD**
In cognitively normal individuals with SCD, pathological hallmarks of AD, such as low CSF Abeta42, amyloid deposition on PET-scans or cortical atrophy, may already be present [40–43]. Low CSF Abeta42 and amyloid deposition on a PET-scan are both associated with an increased risk of clinical progression from SCD to AD dementia [40,41,44]. So far it is not possible to predict with these markers when, and at what pace, an individual with preclinical AD progresses to dementia. Research on novel biomarkers focusses on identifying biochemical processes contributing to AD, and also monitoring disease progression. In our studies we aimed to understand how one of the major genetic risk factors of AD, Apolipoprotein E genotype (APOE) exerts its effects on Alzheimer pathology. APOE encodes for the protein ApoE, which regulates lipid homeostasis in the brain and also supports injury repair [45]. The APOE e4 allele is associated with a higher prevalence of amyloid pathology in cognitive normal elderly, and is associated with an earlier age of onset of dementia [7,45]. APOE is suggested to influence the expression of other apolipoproteins, such as apolipoprotein A1 and apolipoprotein J (clusterin). These latter proteins are involved in lipid homeostasis and possibly also immune response, and protein levels are altered in AD [46,47]. In this thesis we investigated possible influences of apolipoprotein levels in individuals with SCD using ELISA. In search of more easily available biomarkers, such as blood biomarkers, we evaluated apolipoprotein levels in both CSF and plasma. While writing this thesis the new sensitive Single Molecule Array (SIMOA) technology became available [48]. This led to new opportunities to analyze plasma of individuals with SCD in search of novel biomarkers, for example to investigate the more established Abeta42, but also Abeta40, and tau in plasma of individuals with SCD.

**Rationale**

In this thesis we investigated the concept of SCD and biomarkers of AD in the preclinical stage of the disease.

**We aimed**:
1.      To describe characteristics of individuals with SCD in the Subjective Cognitive ImpairmENt Cohort (SCIENCe)
2.      To investigate risk factors of clinical progression from SCD to Mild Cognitive Impairment (MCI) or dementia
3.      To evaluate early biomarkers of AD, and biomarkers of future clinical progression to dementia in initially non-demented elderly with SCD and MCI.

**Outline of this thesis**
**Part 1**. In the first part we describe the Subjective Cognitive ImpairmENt Cohort (SCIENCe), including study design and cross-sectional evaluation of characteristics of the first 150 participants, and a validation of SCD-plus criteria (chapter 2).
**Part 2.** In the second part we investigate risk factors of clinical progression in SCD. In chapter 3 we assess the incidence of dementia for individuals with SCD and the risk of progression to both AD dementia and non-AD dementia in memory clinic and community-based cohorts. Subsequently, we develop biomarker-based personalized risk estimates of clinical progression for individuals with SCD (chapter 4).
**Part 3.** In the third part we evaluate the contribution of novel biomarkers on the risk of AD in individuals with SCD. In chapter 5 we investigate associations between apolipoprotein A1 levels in CSF and plasma and the risk of progression to AD. We subsequently assess APOE genotype effects, and the influence of apolipoprotein E, clusterin and apolipoprotein A1 levels on associations between APOE allele frequency and CSF Amyloid-beta1-42 or tau in chapter 6. In chapter 7 we look at plasma Amyloid-beta1-40 and Amyloid-beta1-42 in individuals with SCD using the SIMOA technique. We test the added value of plasma amyloid in predicting the risk of progression from SCD to AD. We conclude this thesis with a discussion of overall findings and suggestions for future research in chapter 8.

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# CHAPTER 2

## Subjective Cognitive Impairment Cohort (SCIENCe): study design and first results

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Van Berckel B.N.M., Scheltens P., Sikkes S.A.M., Van der Flier W.M.

**ABSTRACT**

**Introduction**: We aimed to (1) describe the Subjective Cognitive Impairment Cohort (SCIENCe) study design, (2) cross-sectionally describe participant characteristics, and (3) evaluate SCD-plus criteria.
**Methods**: SCIENCe is a prospective cohort study of SCD patients. Participants undergo extensive assessment, including CSF collection and optional amyloid PET scan, with annual follow-up. Primary outcome measure is clinical progression.
**Results**: Cross-sectional evaluation of the first 151 participants (age 64±8, 44%F, MMSE 29±2) showed that 28 (25%) had preclinical AD (amyloid status available: n=114 (75%)), 58 (38%) had subthreshold psychiatry, and 65 (43%) had neither. More severe subjective complaints were associated with worse objective performance. SCD-plus criteria age≥60 (OR (95%CI) 7.7 (1.7-38.9)) and APOE e4 (OR 4.8 (1.6-15.0)) were associated with preclinical AD.
**Discussion**: The SCIENCe study confirms that SCD is a heterogeneous group, with preclinical AD and subthreshold psychiatric features. We found a number of SCD-plus criteria to be associated with preclinical AD. Further inclusion and follow-up will address important questions related to SCD.

## INTRODUCTION

Alzheimer´s disease (AD) develops gradually, and the first pathophysiological changes occur decades before a diagnosis of dementia [1,2]. Research interest is shifting to increasingly earlier stages, as the origin of AD and keys to treatment probably lie in prevention of progression to full-fledged disease. Preclinical AD is defined as an asymptomatic stage of AD, in which AD biomarkers are aberrant, but clinical symptoms of objective cognitive decline are not present [3]. Subjective cognitive decline (SCD) refers to the experience of cognitive decline, without formal deficits on neuropsychological testing, nor any other neurological or psychiatric diagnosis explaining cognitive complaints [4]. The subjective experience of cognitive decline has been suggested to be one of the first symptoms of AD, and patients with SCD have an increased risk of progression to MCI or dementia, especially when complaints are reported by both patient and informant [5–10]. In cognitively normal individuals with SCD, biomarkers of AD can already be aberrant, such as low CSF Amyoid-beta1-42, increased amyloid deposition on PET scans and thinner medial temporal cortex [11–14]. However, the sequence of neurodegenerative changes eventually leading to AD may vary amongst individuals, and where to place SCD in these pathological sequences remains to be elucidated.
It is difficult to clinically identify preclinical AD in cognitively healthy individuals experiencing memory complaints. To increase the likelihood of preclinical AD in individuals with SCD, the SCD-I working group has proposed the SCD plus criteria [15]. These criteria include biomarkers such as APOE e4 carriership, but also patient specific features such as predominant self-perceived memory decline and feeling of worse memory performance than others of the same age. The SCD plus criteria have been proposed to facilitate harmonizing SCD research, but they have not yet been prospectively validated.

Even though individuals with SCD on average have an increased risk of AD, most individuals with SCD do not harbor Alzheimer pathology. Alternative potential explanations for the experience of memory complaints in cognitive healthy individuals include subthreshold symptoms of affective disorders, personality features, lifestyle factors or systemic illnesses [16–18]. To evaluate the contribution of different factors related to SCD we have set up the memory clinic based Subjective Cognitive Impairment Cohort (SCIENCe). In this ongoing cohort study we investigate individuals with SCD, without major psychiatric of neurological disorders. Here, we aimed to (i) describe the SCIENCe study design, (ii) cross-sectionally evaluate participants characteristics and factors related to cognitive complaints, and (iii) evaluate recently defined SCD-plus criteria as indicators of preclinical AD.

## METHODS

### Study design and work-up
The Subjective Cognitive Impairment Cohort (SCIENCe) is a prospective cohort study including consecutive patients with SCD presenting at the Alzheimer center of the VU university medical center Amsterdam. Here, we extensively describe the study design of the ongoing SCIENCe study. In addition, we report results based on a selection of cross-sectional data of the first 151 SCIENCe participants.

Inclusion criteria for SCIENCe are a diagnosis of SCD (i.e. cognitive complaints and normal cognition) and age ≥ 45 years. Exclusion criteria are MCI, dementia, major psychiatric disorder (i.e. current depression, personality disorders, schizophrenia), neurological diseases known to cause memory complaints (i.e. Parkinson's disease, epilepsia), HIV, abuse of alcohol or other substances, and language barrier.

All participants have been referred to the memory clinic by their general practitioner, a neurologist or geriatrician in case of a second opinion for evaluation of cognitive complaints. They receive a standardized dementia screening at the memory clinic, including an interview with a neurologist, physical and neurological examination, neuropsychological assessment, as well as routine analyses of blood, CSF and brain magnetic resonance imaging (MRI). After the standardized dementia screening, diagnoses are made in a multidisciplinary consensus meeting. Patients receive a label of SCD when cognitive functioning is normal and when there is no diagnosis of MCI, dementia or any other disease known to cause memory complaints [19]. When subtle symptoms of an underlying psychiatric diagnosis, such as depression, are suspected, patients are evaluated by an experienced psychiatrist to exclude possible formal psychiatric diagnoses as cause of cognitive complaints.

Eligible patients with SCD are invited to participate in SCIENCe. After inclusion in SCIENCe, participants are invited for additional baseline assessments, which are described in detail below. After completion of baseline assessment, patients are invited for an annual follow up visit consisting of clinical evaluation, extensive neuropsychological assessment and questionnaires. At each follow up visit diagnoses are re-evaluated under supervision of a neurologist. Main outcome measures are clinical progression to MCI or dementia and decline in cognitive functioning. If patients progress to MCI or dementia they are offered the possibility to return to routine memory clinic follow up. The SCIENCe work-up is visualized in figure 1.

The local medical ethics committee of the VU University Medical Center approved the study and all patients provide written informed consent for the use of their clinical data and biomaterial in research. All research is conducted in accordance with the Helsinki Declaration of 1975.

SCIENCe inclusion started in June 2014. In the first two years, 243 consecutive individuals aged 45 years or older received a diagnosis of SCD, of which 56 were not eligible for participation and 36 individuals were not interested in participation (figure 2). This led to inclusion of 151 individuals in SCIENCe until the start of data analysis for the current report. In this cross-sectional report of SCIENCE baseline findings, we evaluate these first 151 participants. Further inclusion in SCIENCe and follow up of participants is currently ongoing.
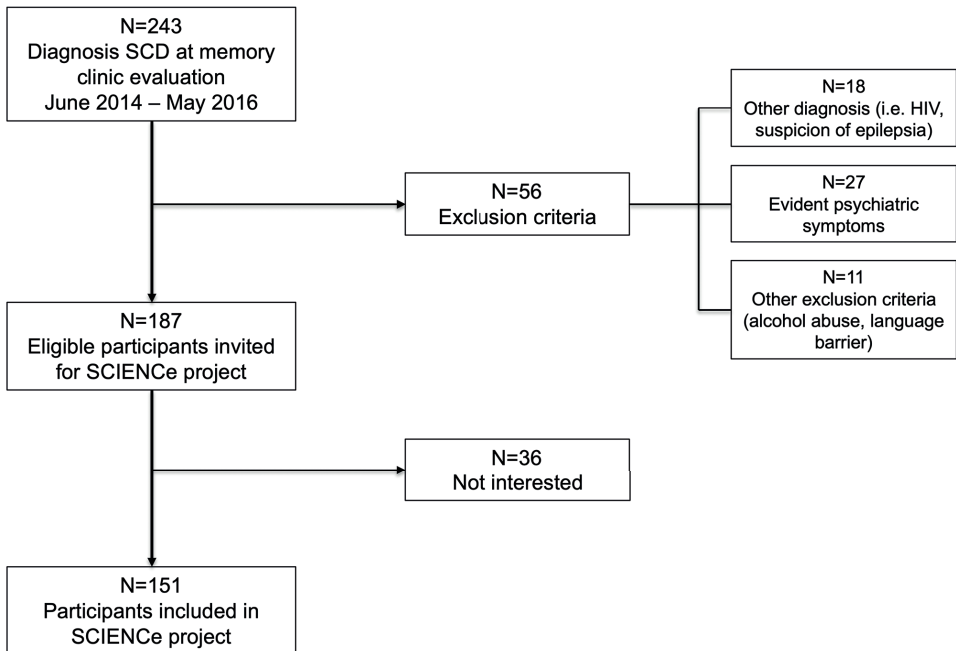


Figure 1. SCIENCe work-up at baseline and annual follow-up study visits. Primary outcome is clinical progression to MCI or dementia.

```
┌─────────────────────────────┐
│   Memory clinic evaluation:  │
│  Diagnosis subjective cognitive │
│            decline           │
│ Clinical evaluation, Neuropsychology, MRI │
│    Blood, DNA, CSF for biobanking │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐
│            T=0              │
│   Inclusion SCIENCe project  │
│ Extra neuropsychology & questionnaires │
│       RNA for biobanking     │
└─────────────────────────────┘
              │
              │        ┌──────────────────────┐
              ├───────▶│ Optional: amyloid/tau  │
              │        │       PET-scan         │
              │        └──────────────────────┘
              │
              │                      ┌──────────────────────────────┐
              │                      │     Clinical evaluation        │
              ▼                      │  Neuropsychological tests +    │
┌─────────────────────────────┐     │        questionnaires          │
│            T=x              │─────│  At T=2: blood + optional CSF  │
│   Annual SCIENCe follow up   │     │    collection for Biobanking   │
└─────────────────────────────┘     └──────────────────────────────┘
              │
              │                      ┌──────────────────────────────┐
              │                      │ If clinical progression to     │
              └─────────────────────▶│        MCI / dementia:         │
                                     │  Evaluation memory clinic      │
                                     └──────────────────────────────┘
```

Figure 2. Flow chart of inclusion of SCIENCe participants evaluated in the current report (n=151). Further inclusion and follow-up is currently ongoing.

## Questionnaires
The supplementary table provides a detailed overview of questionnaires, used to evaluate subjective cognitive decline, mental health, instrumental activities of daily living and lifestyle (i.e. dietary intake, and physical and cognitive activity).

*Subjective cognitive decline*
We use the Dutch translation of the Cognitive Change Index – self (CCI-S) and informant report (CCI-I) (20 questions, range 0 to 80) to assess cognitive function compared to five years ago [13]. Higher scores reflect worse subjective cognitive function. The CCI cut-off for significant cognitive complaints is set at 16/80 [20]. In addition, we use the Subjective Cognitive Functioning (SCF) questionnaire (4 questions, range: -12 to +12) to assess self-experienced cognitive decline over a one-year time period [21]. A SCF score below zero represents decline.

*Psychiatric symptoms*
We use the following questionnaires to evaluate psychiatric symptoms: depressive symptoms (CES-D [22]), anxiety (HADS-A [23]), neuroticism (NPV neuroticism subscale [24,25]), low mastery (Pearlin Mastery scale [26]),

distress and somatization, defined as non-specific physical complaints (4-DKL distress and somatization subscales [24]), and quality of life (EuroQol [27]). For all psychiatric and quality of life questionnaires higher scores reflect worse performance. See supplementary table for cut-offs of questionnaires.

**Neuropsychological evaluation**
All participants received a comprehensive standardized neuropsychological assessment at the regular memory clinic evaluation [19]. As part of SCIENCe baseline investigation, we perform an additional neuropsychological assessment (time between assessments: median 37 days), evaluating cognitive domains: memory, language, attention, executive and visuo-spatial functioning, with a special emphasis on memory, see supplementary table for an overview of the complete SCIENCe test battery. This test battery is repeated at follow up.
In this paper we report on a subset of the neuropsychological assessment. We used the MMSE to assess global cognition [28]. For the memory domain we used the Dutch version of the Rey Auditory Verbal Learning Test (RAVLT) – direct recall (5 trials summed) and delayed recall and cued recall (both >20 minutes) [29]. We used Trail Making Test (TMT) A to evaluate attention, and TMT B to evaluate executive functioning [30]. To evaluate language functioning we used categorical animal fluency.

**Magnetic resonance imaging**
Structural MRI is acquired during the diagnostic visit to the memory clinic using a MR750 (General Electric, Milwaukee, USA), Philips PET/MR (Philips Medical Systems, Best, The Netherlands), or Toshiba Titan (Toshiba Medical Systems, Otawara, Japan). MRI protocol includes isotropic 3D T1-weighted and Fluid Attenuated Inversion Recovery (FLAIR) T2-weighted, and Susceptibility Weighted Imaging (SWI). T1-weighted images are used to estimate hippocampal and normalized brain volumes (NBV) using FIRST and SIENAX with optimized settings (FMRIB software library v5, Oxford, UK) [31,32] is derived from a tissue-type segmentation, using optimized parameters settings, and a scaling factor to normalize for skull size [32]. All registrations are visually inspected for artefacts. All images are read by a neuroradiologist in a standardized fashion. The severity of white-matter hyperintensities (WMHs) using the Fazekas scale is determined on the FLAIR sequence (possible range 0–3), and dichotomized into absent (0–1) or present (2–3). Lacunes are defined as deep lesions (3-15 mm) with CSF-like signal on all sequences. Lacunes are scored as absent or present (≥1 lacune). Microbleeds are defined as small dot-like hypointense lesions on T2-weighted MRI. Microbleed count is dichotomized into absent or present (≥1 microbleed). Here, we present baseline normalized brain volume, bilateral hippocampal volume, WMHs, lacunes and microbleeds. MRI data within one year from SCIENCe inclusion were available for N=116 (77%) participants.

**Biomaterial for biobanking**
Blood (serum and plasma), DNA, and CSF are obtained and stored in our biobank at the department of Clinical Chemistry of the VU University Medical

Center Amsterdam, according to international consensus standard operation procedures [33,34].

*2.5.1 Blood and DNA*

Venous blood (2–6 ml clotted blood for serum and 6 ml EDTA blood for plasma) is processed and stored according to international consensus standard operation procedures. 2–4 ml EDTA whole blood is collected for DNA extraction. After collection plasma and serum samples are centrifuged at room temperature at 2000 x g (min 1,800 x g, max 2,200 x g), aliquoted into 0.5 ml vials and stored at –80◦C.

*2.5.2 RNA*

After inclusion in SCIENCe one PAXgene Blood RNA tube (PreAnalytiX, Qiagen, Venlo, The Netherlands) is collected and without aliquoting stored in the biobank at –80◦C.

*2.5.3 CSF*

CSF is collected from non-fasted subjects. CSF is obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space by a 25-gauge needle and collected in polypropylene tubes.

After collection CSF and plasma samples are centrifuged at room temperature at 2000 x g (min 1,800 x g, max 2,200 x g), aliquotted into 0.5 mL vials and stored at –80°C. A maximum of 2 hours is allowed between collection and freezing [33,34].

**APOE genotyping**

APOE genotyping is performed after automated genomic DNA isolation from 2-4 mL EDTA blood. It is subjected to PCR, checked for size and quantity using a QIAxcel DNA Fast Analysis kit (Qiagen, Venlo, The Netherlands) and sequenced using Sanger sequencing on an ABI130XL. Here, APOE status was available for n=144 (95%). Subjects with at one or two e4 alleles were classified as APOE e4 carriers.

**Cerebrospinal fluid markers**

From the total amount of collected CSF at memory clinic visit, 2.5 mL is used for routine analyses, including leukocyte count, erythrocyte count, glucose concentration, and total amount of protein, and frozen at –20◦C until further analysis of Alzheimer biomarker. Amyloid-beta1-42 (Abeta42), tau and tau phosphorilyzed threonine 181 (ptau) levels are measured using ELISA (Innogenetics-Fujirebio, Ghent, Belgium) at the Neurochemistry Laboratory [35]. Our center cut-off for CSF Abeta42 indicating AD pathology is <640 ug/L [36].

**Positron Emission Tomography (PET) scans**

All participants are invited to participate additionally in an amyloid PET study. Patients are scanned with either [18F]florbetapir (or Amyvid) or [18F]florbetaben (Neuraceg) radiotracer. Before scanning, one cannula is inserted for tracer infusion. For florbetapir, 90 minutes dynamic PET emission scans (PET/CT Ingenuity TF or Gemini TF, Philips Medical Systems, Best, The Netherlands)

are acquired immediately following bolus injection of approximately 370MBq [18F]florbetapir. For florbetaben, 20 minutes static PET emission scans (PET/MR, Philips Medical Systems, Best, The Netherlands) are acquired 90 minutes after a bolus injection of approximately 250MBq [18F]florbetaben. All PET scans are visually read by a nuclear medicine physician. For the current manuscript, PET-scans were available for 105/151 (69%) participants.

**Amyloid status**
Information on amyloid status was available for 114 (75%) particpants (PET only N=38 (25% of total), CSF only N=9 (6%), CSF&PET N=67 (44%)). Amyloid status could be determined if: (i) CSF and/or amyloid PET were performed within one year of baseline visit, or (ii) if repeated amyloid measurements were concordant before and after baseline (i.e. both negative or both positive). There were seven cases with discordant PET/CSF results. In all seven cases, CSF Abeta42 was above the cut-off of 640ug/L (range 645 – 881ug/L), but amyloid PET was positive; we considered these cases as amyloid positive.

**Categorization of participants according to concomitant symptoms**
In this cross-sectional report of SCIENCE baseline findings, we categorized SCIENCe participants into categories based on the presence of preclinical AD and/ or subthreshold psychiatry, as potential factors associated with SCD [4]:
1. Preclinical AD: Amyloid positive individuals based on PET and/or CSF amyloid (see paragraph 2.9) were classified as preclinical AD.
2. Subthreshold psychiatry: Individuals with one or more questionnaires indicative of subthreshold symptoms of depression, anxiety, neuroticism, low mastery, distress or somatization, were classified as subthreshold psychiatry (see supplement for overview of questionnaires and cut-offs used). Fulfillment of clinical criteria for a formal psychiatric diagnosis was an exclusion criterion for SCIENCe, hence psychiatric symptoms measured with the questionnaires were subthreshold. When participants were amyloid positive, but also had subthreshold psychiatric symptoms they were classified in the preclinical AD group. Amyloid status was not available in the subthreshold psychiatry category for 21 of 58 cases (36%).
3. Undetermined: When participants were neither amyloid positive, nor was there any indication of subthreshold psychiatric symptoms, they were classified in the undetermined category. Amyloid status was not available in the undetermined category for 16 of 65 patients (25%).

**SCD-plus criteria**
The SCD-plus criteria refer to specific features of SCD associated with an increased likelihood of preclinical AD [4]. The SCD-plus criteria are: (1) subjective decline in memory, rather than other domains of cognition (in our study defined as 'memory decline present' as evaluated in the SCF questionnaire), (2) onset of SCD within the last 5 years, (3) age at onset of SCD ◻60 years, (4) concerns (worries) associated with SCD, (5) feeling of worse performance than peers (here operationalized with a specific question in the

CCI questionnaire), (6) confirmation of perceived cognitive decline by an informant (here operationalized as a CCI informant report score above cut-off of significant symptoms (>16)), and (7) APOE e4 carriership. We evaluated the SCD-plus criteria with the exception for criterion worries associated with SCD (4), which we considered present in all, since participants all visited our memory clinic because of cognitive complaints.

**Statistical analyses**
Data were analyzed using IBM SPSS Statistics, version 22 (IBM, Armonk, NY). We assessed baseline features of the study population and evaluated differences between participant categories (preclinical AD, subthreshold psychiatry or undetermined), using chi-squared tests or ANOVA, adjusted for age and gender, as appropriate, followed by post-hoc analyses. We used univariate linear regression analyses to assess associations between cognitive complaints (CCI-S, CCI-I and SCF) and neuropsychological test scores, adjusted for age and gender. Furthermore, we evaluated the prevalence of the SCD-plus criteria in participants with available amyloid status. Subsequently, we used logistic regression to investigate the associations of SCD-plus criteria with the risk of preclinical AD. First, we performed univariate models with each SCD-plus criterion separately (model 1). Then, we constructed model 2 as a multivariate model with backward stepwise selection with the 6 available SCD plus criteria. We considered $p<0.05$ significant.

## RESULTS

### Baseline demographics
At baseline the first 151 SCIENCe participants were on average 64±8 years old (range 45-84 years), and 67 (44%) were female (table 1). Participants received on average 12±3 years of education, and 76 (54%) had a family history of dementia. 55 participants (38%) were APOE e4 positive (APOE e4 status available for n=144 (95%)).

### Self-report of SCD
We cross-sectionally assessed report of subjective cognitive functioning compared to one year ago (SCF self-report) and five years ago (CCI; both self- and informant-report; Table 1)). Over the preceding five-year time period 146 (97%) participants reported cognitive decline (CCI-S), of which 89 (60%) reported substantial decline. Over a one-year time period (SCF) 104 (69%) participants reported substantial cognitive decline. Adjusted for age, gender and education, higher CCI-S was associated with worse SCF (standardized Beta -.40, p<0.001), and CCI-S was also associated with CCI-I (sBeta 0.48, p<0.001; table 2). In addition, we found that higher self-report of subjective cognitive functioning (CCI-S and SCF) was associated with worse quality of life (sBeta -.34; sBeta .25; both p<0.05). Furthermore, higher CCI (both self and informant) were associated with worse performance on cognitive tests (table 2), while there were no associations between SCF and objective measures of cognition.

### SCD groups
When we attempted to categorize participants according to the presence of preclinical AD and/or subthreshold psychiatric symptoms, we found 28 (25% of 114 participants with known amyloid status, and 18% of total sample) with preclinical AD. Higher age was associated with an increased risk of preclinical AD (Odds ratio 1.14 (95% CI 1.06-1.22); figure 3).
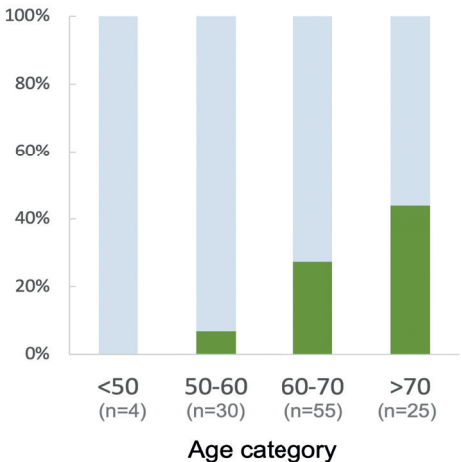


Figure 3. Percentage of amyloid positivity per decade in SCD participants (n=114)

In the remaining sample, 58 (38%) participants reported subthreshold psychiatric symptoms on one or more questionnaires. Of these participants 21% had subthreshold symptoms in the affective cluster, for example depressive (11%) and/or anxiety (13%) symptoms. Roughly one out of three (31%) had distress and/or somatization related symptoms, and in 27% there was an indication of symptoms of neuroticism and/or low mastery. In addition, eight of 28 (29%) patients in the preclinical AD category also had subthreshold psychiatric symptoms. The largest group of SCD (n=65 (43%)) had neither evidence of amyloid, nor of subthreshold psychiatric symptoms (undetermined category).

Compaing these three SCD groups, participants with preclinical AD were on average older than individuals in the subthreshold psychiatry (p<0.001; table 1) and undetermined category (p<0.05). Participants with preclinical AD more frequently had a family history of dementia than subthreshold psychiatry, and they were more frequently APOE e4 carrier than the other two groups (all p<0.01). There were no differences in gender, education or MRI measures between groups. Self-reported cognitive decline was higher in participants with subthreshold psychiatry than in the undetermined category, with preclinical AD in between (both p<0.01). Results were similar for informant reported cognitive decline. Reported quality of life was lower in the subthreshold psychiatry group than in the undetermined category (p=0.002), with preclinical AD in between. Comparing objective cognitive performance between groups, the group with subthreshold psychiatry performed worse on the TMT-A compared to preclinical AD and undetermined groups (all p<0.05). Also, subthreshold psychiatry performed worse on the TMT-B than the undetermined group (p<0.05), but there were no differences in other cognitive tests, see Table 1.

**SCD-plus criteria and the risk of preclinical AD**
Univariate logistic regression analyses showed that SCD-plus criteria 'age > 60' (OR 7.7 (95% CI 1.7-34.6)) and 'APOE e4 carriership' (OR 5.0 (2.0-12.8)) were associated with an increased risk of preclinical AD (figure 4), whereas 'memory specific decline', 'onset of complaints within 5 years', 'worse performance than other of the same age', and 'informant reports decline' were not (table 3). In a multivariate stepwise model, APOE e4 carriership (OR 6.2 (1.7-22.2)) and age > 60 (OR 3.8 (1.7-20.4)) remained independently associated with preclinical AD.
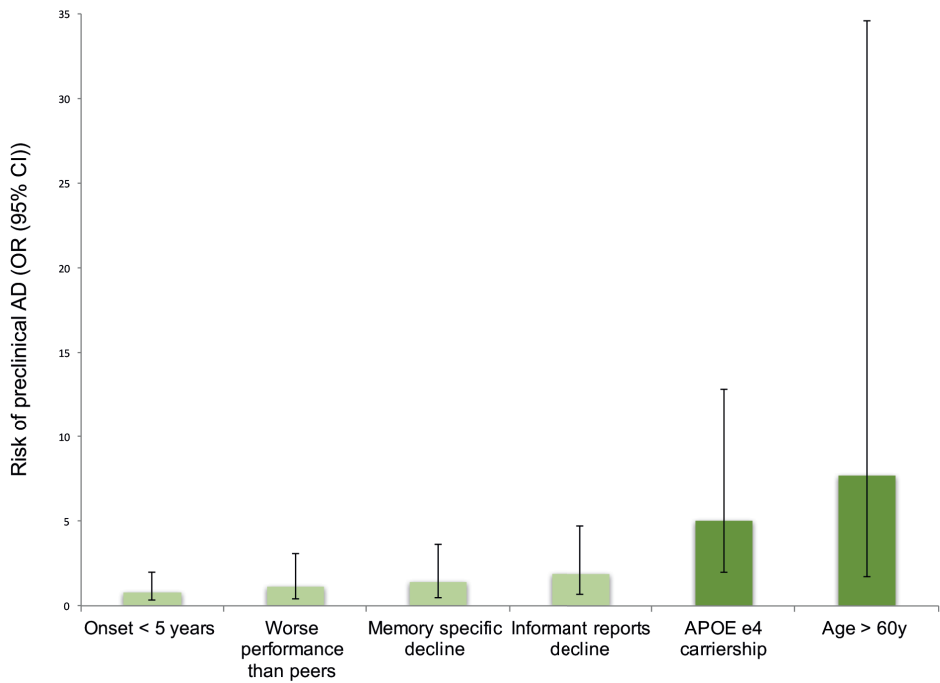
Figure 4. SCD-plus criteria and the risk of preclinical AD for each SCD-plus criterion (Odd ratios (95% Confidence Interval)) in participants with available amyloid status (n=114)

## DISCUSSION

The SCIENCe project aims to investigate factors potentially related to SCD. Cross-sectional evaluation of the first 151 cognitively normal participants with SCD revealed a heterogeneous group, with preclinical AD in one fifth to one quarter of participants, and subthreshold psychiatric symptoms in more than one third of participants, while the largest group of participants did not have evidence of either. We found that higher report of SCD was associated with lower quality of life, and also with worse cognitive performance. Finally, SCD plus criteria age◻60 and APOE e4 carriership were associated with an increased risk of preclinical AD, defined by amyloid positivity on either PET or in CSF.

We measured the degree of subjective complaints with the short SCF questionnaire and used the CCI for more in depth evaluation [13,21]. Almost all participants reported cognitive decline, which seems substantially higher than in the general population [37], and could be a reflection of our cohort with individuals actively seeking medical evaluation in a memory clinic because of these cognitive complaints. A small minority of 3% did not report any complaints, potentially explained by the fact that participants filled in the questionnaires after a thorough memory clinic evaluation, with reassurance of normal cognitive functioning.
We found that higher report of cognitive complaints was associated with worse quality of life, suggesting that subjective complaints have a negative effect on a general feeling of wellbeing. On the other hand, we cannot exclude reverse causality, as worse quality of life may also affect the subjective appreciation of one's (cognitive) abilities [38]. Furthermore, we found that higher report of cognitive complaints on the CCI (both self and informant) was associated with worse objective cognitive performance in our cognitive normal sample with SCD, which is in line with literature on the CCI and objective performance [39]. Although self-report of SCD has been associated with future cognitive decline [5,8], and also has been suggested to be more sensitive for subtle decline than informant report in the very earliest stages of cognitive decline, earlier cross-sectional associations have not been consistent [11,40–42]. This could be a result of the use of different SCD measures [43]. Indeed, we found no significant associations between objective cognition and SCF, which measures cognitive complaints over a shorter period of time and consisting of four questions only, in contrast to the observed associations with the CCI.

Cognitive complaints in cognitively normal individuals were previously found to have a broad range of associated symptoms, varying from distress to affective disorders, systemic illnesses, and preclinical AD [11,12,16,17,40]. In the current paper we evaluated the prevalence of preclinical AD and subthreshold psychiatric features as potential factors associated with the occurrence of SCD [4]. We observed that 25% of participants with available amyloid status had preclinical AD, and amyloid positivity increased with age. Although we did not make a formal comparison, percentages of amyloid positivity per decade seem

somewhat higher in our cohort than in individuals with SCD in a recent large meta-analysis investigating amyloid prevalence in non-demented elderly [44].

In our sample, 38% of participants experienced subthreshold psychiatric symptoms on one or more domains. These symptoms were labeled subthreshold, since individuals with a clear psychiatric diagnosis, such as major depression, were not included. The group with subthreshold psychiatric symptoms reported more cognitive complaints than the group with preclinical AD. We evaluated six psychiatric features which have been previously associated with cognitive complaints in individuals with SCD, and might provide an alternative explanation for the subjective experience of decline [16,17,40,42,45]. On the other hand, several of these psychiatric features, such as depressive symptoms, anxiety, neuroticism and distress, have also been associated with preclinical AD [46–52], and indeed, we also saw the co-occurrence of preclinical AD and subthreshold psychiatric symptoms in 8 of 28 cases. We are currently following all participants to study clinical progression in these different groups.

For 43% of the remaining SCIENCe participants, we found neither preclinical AD nor subthreshold psychiatry. Individuals in the undetermined category had less cognitive complaints than the other two categories, both reported by themselves and by the informant. Nonetheless, each of these patients was referred to the memory clinic for evaluation of complaints. In the undetermined category we found a higher prevalence of family history of dementia than in the subthreshold psychiatry category, similar to preclinical AD. Perhaps anxiousness related to family history of dementia, rather than the actual experience of cognitive decline, could be a reason to visit the memory clinic for evaluation [53].

To facilitate harmonization of SCD research, the international SCD Working Group (SCD-I) have published a conceptual framework on SCD research, which included the SCD-plus criteria as determinants of preclinical AD [4]. This is the first time the SCD-plus criteria were comprehensively evaluated in a clinical setting. We found that SCD-plus criteria age¤60 and APOE e4 carriership were associated with an increased risk of preclinical AD, which is in line with literature [11,44,54]. The four other SCD-plus criteria we evaluated were not associated with preclinical AD in our cohort. There was a trend for an increased risk of preclinical AD when the informant reported significant decline, but results were not significant. The lack of association between informant report and preclinical AD is in contrast with a previous study showing an association between these factors [55]. This contrast could possibly be explained by differences in informant report measurement methods, as well as, differences in sample size between the previous study and ours. Since informant report seems to be a better predictor of future cognitive decline than patient report [7,10,56], future longitudinal evaluation of SCIENCe participants and extension of sample size may reveal further relations. Furthermore, criteria 'worse performance than others of the same age' and 'memory specific decline' were not associated

with an increased risk of preclinical AD, which is in contrast to previous studies indicating both concepts to be associated with preclinical AD [11,57]. We used questions from the CCI and SCF to assess these topics (respectively feeling of worse performance than others (yes/no) and how do you evaluate your memory function compared to one year ago (stable/decline)). For these two SCD-plus criteria differences between results may be caused by methodological variation in SCD measurements, which are known to result in great variation between studies [43]. Criterion 'onset of symptoms within 5 years' did not alter the risk of preclinical AD in our cohort. To our knowledge this is the first study evaluating the association between onset of symptoms within 5 years and preclinical AD, whereas others evaluated the risk of future cognitive decline in relation to onset of symptoms, without taking into account preclinical AD [58–60].

Limitations of the study include the availability of amyloid status in the cohort for 114 of 151 participants. Because of the availability of amyloid status, participants that are now classified in the subthreshold or undetermined category may have preclinical AD of which we are unaware, since we hierarchically first included participants in the preclinical AD group, followed by categorization of the remaining participants (amyloid status negative or unknown) in the other two groups. Strengths of the study include the highly standardized assessment of a broad range of factors potentially related to SCD, including various biomarkers, as well as repeated collection of blood and CSF for biobanking to be able to evaluate biomarkers longitudinally.

In the light of a disease evolving over decades, longitudinal evaluation seems necessary to assess if, and when, those with and without preclinical AD eventually show progression to MCI or dementia. In SCIENCe we aim to evaluate which factors predict progression, but also which factors are protective of future decline. Discriminating preclinical AD from the 'worried well' seems especially important as anti-amyloid therapies targeting early stages of AD appear a realistic possibility in the nearby future. Furthermore, assessment of factors other than preclinical AD contributing to SCD may be of importance, since also non-pharmacological interventions seem to be of added value in individuals with SCD [62].

## CONCLUSIONS
In summary, this first cross-sectional evaluation of SCIENCe participants revealed that SCD is a heterogeneous group, with besides preclinical AD also subthreshold psychiatric features. We found that subjective report of decline was associated with objective measures. Furthermore, we found a number of SCD-plus criteria to be associated with preclinical AD. Further inclusion and follow-up will address important questions related to SCD.

**Table 1.** Demographic features of the study population

| | | N | Total group (n=151) | Preclinical AD (n=28) | Subthreshold psychiatry (n=58) | Undetermined (n=65) | P |
|---|---|---|---|---|---|---|---|
| **Demographics** | Age | 151 | 64±8 | 69±7[b] | 62±8[a] | 64±8[a] | .002 |
| | Gender (female) | 151 | 67 (44) | 11 (39%) | 27 (47%) | 29 (45%) | Ns |
| | Education, years | 148 | 12±3 | 13±3 | 12±3 | 12±2 | Ns |
| | Family history dementia | 140 | 76 (54) | 18 (75%)[b] | 20 (36%)[a] | 38 (62%)[b] | .002 |
| | APOE e4 carrier | 144 | 55 (38) | 17 (65%)[b] | 17 (30%)[a] | 21 (34%)[a] | .007 |
| | | | N above cut-off | | | | |
| **Subjective Cognitive Decline** | SCF (1y change) self-report | 150 | -1.65±2.98  104 (69%) | -2.0±2.3 | -2.4±3.2 | -0.8±2.9[b] | .004 |
| | CCI (5y change) self-report[h] | 148 | 21.8±14.3  89 (60%) | 21.4±13.2[b] | 27.8±15.2[a] | 16.7±11.8[b] | .000 |
| | CCI (5y change) informant[h] | 127 | 19.4±17.1  62 (49%) | 19.8±13.9[b] | 26.4±19.1[a] | 13.8±14.8[b] | .000 |
| **Mental health questionnaires** | Quality of Life | 149 | 76±15 | 79±12 | 71±16 | 80±15[b] | .012 |
| | Depressive symptoms[h] | 150 | 8.3±6.4  17 (11%) | 7.0±4.6 | 12.1±7.3 | 5.5±4.1 | .000 |
| | Anxiety[h] | 150 | 4.0±2.9  13 (13%) | 4.1±2.6 | 5.6±3.1 | 2.4±1.9 | .000 |
| | Distress[h] | 150 | 6.7±5.9  34 (22%) | 4.6±4.6 | 11.1±6.3 | 3.6±2.9 | .000 |
| | Somatization[h] | 151 | 6.3±5.3  31 (21%) | 4.6±3.7 | 10.3±5.7 | 3.5±2.8 | .000 |
| | Neuroticism[h] | 145 | 6.6±5.5 | 5.2±3.8 | 10.1±6.4 | 4.0±2.8 | .000 |
| | Low mastery[h] | 140 | 10.5±3.9 | 10.0±3.0 | 12.8±4.0 | 8.5±2.7 | .000 |

| Cognition | | | | | | | |
|---|---|---|---|---|---|---|---|
| | MMSE | 151 | 28.6±1.2 | 28.4±1.3 | 28.5±1.2 | 28.9±1.2 | .031 |
| Memory domain | RAVLT immediate recall | 149 | 44.3±9.0 | 43.4±8.7 | 44.0±9.0 | 44.6±9.2 | Ns |
| | RAVLT delayed recall | 149 | 9.0±2.9 | 8.5±2.9 | 9.1±3.0 | 9.2±2.9 | Ns |
| | RAVLT cued recall | 149 | 28.7±1.6 | 28.7±1.5 | 28.5±2.3 | 28.8±1.4 | Ns |
| Attention | TMT A[h] | 148 | 34.4±12.8 | 33.4±12.2[b] | 37.5±14.9[a] | 32.1±10.5[b] | .014 |
| Executive functioning | TMT B[h] | 147 | 82.1±33.2 | 79.5±28.9 | 89.8±41.0 | 76.2±25.8[b] | .058 |
| Language | Animal fluency | 147 | 23.2±5.2 | 22.8±4.9 | 23.1±5.1 | 23.5±5.6 | Ns |
| MRI | Normalized brain volume, ml | 116 | 1399±79 | 1366±75 | 1407±81 | 1406±79 | Ns |
| | Bilateral hippocampal volume, ml | 116 | 9.9±1.3 | 10.0±1.4 | 9.9±1.3 | 9.8±1.1 | Ns |
| | White-matter hyperintensities (present) | 116 | 10 (9) | 2 (9) | 2 (4) | 6 (13) | Ns |
| | Lacunes (>0) | 115 | 3 (3) | 1 (4) | 2 (5) | 0 (0) | Ns |
| | Microbleeds present (>0) | 112 | 19 (17) | 7 (30) | 4 (10) | 8 (17) | Ns |

Table 1. Unadjusted results are presented as mean±SD or N (%). Differences between groups were assessed using age, gender and education adjusted ANOVA or Chi-squared tests. a=$p<0.05$ difference with preclinical AD, b=$p<0.05$ difference with subthreshold psychiatry, h=higher scores reflect worse performance or more symptoms.

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**Table 2.** Associations between subjective and objective cognitive measures

|  | SCF | CCI-self | CCI-informant |
|---|---|---|---|
| CCI-self [h] | -.39** | | |
| CCI-informant [h] | -.19* | .49** | |
| EuroQol | .25* | -.33** | -.15 |
| MMSE | .14 | -.30** | -.10 |
| RAVLT immediate recall | .01 | -.21* | -.15 |
| RAVLT delayed recall | .03 | -.16 | -.04 |
| RAVLT cued recall | -.12 | -.23* | -.17* |
| TMT A [h] | -.06 | .12 | .17 |
| TMT B [h] | -.17 | .23* | .26* |
| Animal fluency | .15 | -.15 | -.14 |

Associations are presented as standardized A1:D12 adjusted for age, gender and education. * p<0.05, ** p<0.001, h=higher scores reflect worse cognitive performance. SCF = subjective cognitive functioning questionnaire (lower scores indicate more complaints), CCI = cognitive change index (higher scores indicate more complaints), MMSE = Mini-Mental State Examination, RAVLT = Rey Auditory Verbal Learning Test, TMT = Trail Making Test.

**Table 3.** SCD-plus criteria and the risk of preclinical AD in individuals with available amyloid status (n=114)

| Predictor | Data availability (N) total n=114 | Prevalence of SCD-plus criteria | | Risk of preclinical AD | |
|---|---|---|---|---|---|
| | | Preclinical AD n=28 | Amyloid negative n=86 | Univariate model | Multivariate stepwise model |
| Memory specific decline | 94 | 13 (59%) | 37 (51%) | 1.4 (0.5-3.6) | - |
| Onset <5 years | 111 | 12 (46%) | 43 (51%) | 0.8 (0.3-2.0) | - |
| Age > 60y | 114 | 26 (93%) | 54 (63%) | 7.7 (1.7-34.6) | 3.8 (1.7-20.4) |
| Experience of worse performance than others | 90 | 13 (65%) | 44 (63%) | 1.1 (0.4-3.1) | - |
| Informant reports decline | 97 | 15 (60%) | 32 (44%) | 1.9 (0.7-4.7) | - |
| APOE e4 carriership | 110 | 17 (65%) | 23 (27%) | 5.0 (2.0-12.8) | 6.2 (1.7-22.2) |

Prevalence of each SCD-plus criterion in individuals with and without preclinical AD, presented as N(%), within cases with amyloid status available (n=114). Risk of preclinical AD separately (univariate models) for each SCD-plus criterion and independent predictors of preclinical AD in a multivariate stepwise model in SCIENCe participants with available amyloid status (n=114), results presented as Odds Ratio (95% Confidence Interval) (OR (95% CI)).

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**Supplementary table.** Standardized tests and questionnaires used in the SCIENCe project (2017)

| Category | Domain | Name of test or questionnaire | Cut-off |
|---|---|---|---|
| Clinical evaluation | Anamnesis | Anamnesis, evaluation of complaints | |
| | | Medical history | |
| | | Family history | |
| | | Alcohol intake, smoking, drugs | |
| | Physical measurements | Weight, height, waist | |
| | | Blood pressure | |
| Subjective cognitive decline | Self-perceived decline | Cognitive change index – self-report, functioning compared to 5 years ago [1] | < 0 |
| | | Subjective cognitive functioning, change over 1 year [2] | ≥ 16 |
| | Informant report | Cognitive change index – informant report [1] | ≥ 16 |
| Quality of life | | EuroQol, visual analogue scale [3] | |
| Mental health questionnaires | Depressive symptoms | Center for Epidemiologic Studies Depression Scale (CES-D) [4] | ≥ 16 |
| | Anxiety | Hospital anxiety and depression scale (HADS) – anxiety subscale [5] | ≥8 |
| | Distress | Four Dimensional Symptom Question-naire – distress subscale [6] | ≥10 |
| | Somatization | Four Dimensional Symptom Question-naire – somatization subscale [6] | ≥10 |
| | Neuroticism | Dutch Personality Inventory – Neuro-ticism scale [7] | 80th percentile |
| | Mastery | Pearlin Mastery psychological coping scale [8] | 80th percentile |
| Neuro-psychological tests | Global cognition | Mini-mental state examination [9,10] | |
| | Memory | Visual association task (VAT) A&B [11] | |
| | | Dutch version of the Rey Auditory Verbal Learning Test (RAVLT) – direct recall; delayed recall and cued recall after 20 min [12,13] | |
| | | Visual Reproduction I&II (Wechsler Memory Scale (WMS) IV) [14] | |

| | | Story immediate and delayed recall (Rivermead Behavioral Memory Test (RBMT) III, Dutch version) [15,16] |
|---|---|---|
| | | Rey Complex Figure delayed recall (3 min) [17] |
| | Attention | Digit span forward [18] |
| | | Trail Making Test (TMT) A [19] |
| | | Stroop word naming (I) [16,20] |
| | | Stroop colour naming (II) [16,20] |
| | Executive functioning | Trail Making Test (TMT) B [19] |
| | | Digit span backwards [18] |
| | | Stroop colour-word test [16,20] |
| | | Frontal Assessment Battery (FAB) [21] |
| | | Letter Fluency Test (version D-A-T) [22] |
| | Language | Category fluency animals [16] |
| | | Boston Naming Test (30 items) [23–25] |
| | | Visual association test – 'naming' [11] |
| | Visuo-spatial functioning | Fragmented letters (VOSP: Visual Objective and Space Perception) [26] |
| | | Number location (VOSP) [26] |
| | | Dot Counting (VOSP) [26] |
| | | Rey Complex Figure Copy task [17] |
| Activities of daily living | IADL | Amsterdam IADL questionnaire [27] |
| Lifestyle questionnaires | Dietary intake | EETscore [28] |
| | Physical activity | Physical Activity Scale for the Elderly [29] |
| | Cognitive activity | Cognitive Activity Scale [30] |

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# CHAPTER 3
## Subjective cognitive decline and rates of incident Alzheimer's disease and non-Alzheimer's disease dementia

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## ABSTRACT

**Introduction**: In this multi-center study on SCD in community based and memory clinic settings, we assessed (i) incidence of AD and non-AD dementia, and (ii) determinants of progression to dementia.

**Methods**: 11 cohorts provided 2978 participants with SCD and 1391 controls. We estimated dementia incidence and identified risk factors using Cox proportional hazards models.

**Results**: In SCD, incidence of dementia was 17.7 (95%Poisson CI 15.2-20.3)/1000 person-years (AD: 11.5(9.6-13.7), non-AD: 6.1(4.7-7.7)), compared to 14.2 (11.3-17.6) in controls (AD: 10.1(7.7-13.0), non-AD: 4.1(2.6-6.0)). The risk of dementia was strongly increased in SCD in a memory clinic setting, but less so in a community based setting. In addition, higher age (HR 1.1(95%CI 1.1-1.1)), lower MMSE(0.7(0.66-0.8)), and APOE e4(1.8(1.3-2.5)) increased the risk of dementia.

**Discussion**: SCD can precede both AD and non-AD dementia. Despite their younger age, individuals with SCD in a memory clinic setting have a higher risk of dementia than those in community based cohorts.

## INTRODUCTION

Neurodegenerative changes, eventually leading to dementia due to Alzheimer's disease (AD), begin to accumulate  approximately twenty years before clinical symptoms appear [1]. With the lack of curative treatment for dementia due to AD, research is moving towards the prodromal and preclinical stages of AD [2]. Subjective cognitive decline (SCD) refers to the subjective experience of cognitive decline, without objective impairment on cognitive assessment [3]. Compared to individuals without SCD, cognitively normal elderly experiencing complaints have an increased risk of subsequent objective cognitive decline, i.e. progression to Mild Cognitive Impairment (MCI) and dementia [4-9]. Therefore, SCD has been suggested to be a possible first symptomatic expression of preclinical AD [2,3].

The conceptual framework on SCD published by the international Working Group (SCD-I) has a focus on SCD as an early harbinger of AD, proposing the SCD-plus criteria as potential risk factors for preclinical AD[3]. However, SCD may also precede other dementia subtypes with a gradual onset, such as vascular dementia (VaD), dementia with Lewy Bodies (DLB), or frontotemporal dementia (FTD).  Although individuals with SCD have an increased risk of dementia [4-9], the incidence rate of progression from SCD to AD dementia, and especially non-AD dementia, have not been estimated before.

For cognitively normal individuals, risk factors of dementia include higher age, lower education and APOE e4 status [10,11]. Whether these risk factors influence the risk of progression to AD and non-AD dementias in patients with SCD in a similar way, remains to be further investigated. The aim of our multi-center study including both memory clinic and community based cohorts of SCD, was to estimate incidence rates of dementia, both for AD and non-AD.

## METHODS

This collaborative project was initiated during a public meeting of the Subjective Cognitive Decline Professional Interest Area (PIA) during the Alzheimer's Association International Conference in 2015, which was facilitated by the International Society to Advance Alzheimer's Research and Treatment (ISTAART).

### Setting and recruitment

Eleven cohorts provided data, see table 1 for an overview of participating cohorts and the number of subjects included. Across studies, there are differences in operationalization of SCD. We deliberately took the case definition of each study as starting point, table 1 provides information on center-specific operationalization of SCD. Cohorts were defined as memory clinic setting when patients were referred to the memory clinic by a physician, or actively approached the respective center for evaluation. Cohorts were labelled as community setting when the study was population based, for example if recruitment was organized via standardized evaluation of eligible participants in a pre-defined district, or when participants were recruited by active (media) appeal.

### Participants

We included SCD participants in the analysis if (i) the participant reported subjective experience of cognitive decline on one or more cognitive domains; (ii) the participant had normal baseline cognition, defined by results of cognitive assessment within normal ranges (center-specific), and criteria for MCI or any dementia were not met [12–15], and (iii) had at least one follow up assessment (>8 months from baseline) with repeated evaluation of diagnosis. Controls were provided by the same cohorts, but did not endorse inclusion criterion (i). Exclusion criteria were: MCI, dementia, alcohol or substance abuse, or any psychiatric or neurological disease possibly causing memory complaints (i.e. epilepsy, Parkinson's Disease). In sum, 11 cohorts provided 5521 participants; 2978 cases with SCD and 1391 controls without SCD, see figure 1 for an overview of participant selection.

### Outcome measure

The main outcome measure was progression to dementia. Definitions and criteria of specific dementias used in each cohort are provided in the supplement or study design reports. Besides dementia due to Alzheimer's Disease [13,16], we evaluated the following non-AD dementias: vascular dementia [17], frontotemporal dementia [14], and dementia with Lewy Bodies [15]. Other less frequent neurodegenerative causes of dementia, such as corticobasal syndrome or progressive supranuclear palsy, were classified as 'dementia other'.
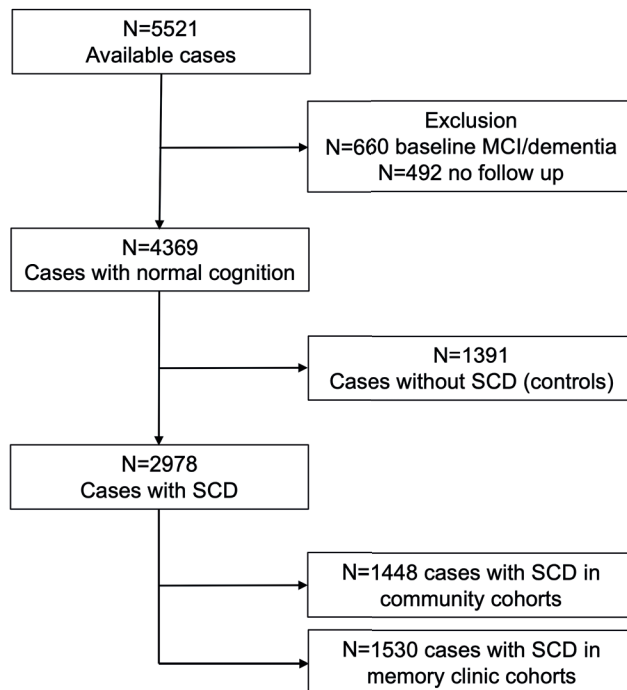
Figure 1. Flowchart of participant selection

## Demographic features of the study population
Sociodemographic features and cognition were assessed in each cohort. Here, we report on age, sex, education, global cognition and APOE e4 carrier status. Information on years of education was available for 2142 (71.9%) participants.

## Cognition
Cognitive function was screened with the MMSE [18], and available for 2928 (98.3%) participants. APOE genotyping was performed according to local procedures, and available for 2417 (81.2%). We dichotomized APOE e4 status (0 e4 alleles vs. 1 or 2 e4 alleles).

## Statistical analyses
Data were analyzed using SPSS (version 22, IBM, Armonk, NY, USA) and Stata 15 (Stata Statistical Software: Release 15, StataCorp LP). We evaluated baseline characteristics, and assessed differences between memory clinics and community cohorts using linear mixed models (continuous variables) or generalized estimating equations (dichotomous variables), taking into account random center effects.
We calculated incidence rates of dementia per 1000 person years with accompanying 95% Poisson confidence intervals, and incidence rates of AD and non-AD dementia separately.

We studied the effect of age, sex, MMSE, number of education years, APOE e4 carrier status and recruitment setting (memory clinic vs. community cohort) on the risk of dementia by using shared-frailty Cox proportional hazards models, taking into account within-group center effects. We conducted simple and multiple Cox regression models and accounted for residual variation in progression risk among studies by including a center-specific random effect. To evaluate whether effects of MMSE and education were generalizable between centers, we added mean MMSE and mean number of education years per center as variables. Finally, we added interaction effects of recruitment setting and variables age, sex, MMSE, and number of education years. We repeated the analyses stratified for AD and non-AD dementia.

For visualization, we constructed Kaplan Meier curves of progression to dementia in general, and for dementia due to AD and to non-AD per decade of age. When calculating the risk of AD, cases progressing to non-AD dementia were censored and vice versa. P<0.05 was considered to be significant.

## RESULTS

### Demographics

Table 2 shows the baseline demographic features of the study population. Individuals with SCD in memory clinic cohorts were on average 10 years younger and they were less often female, had more years of education, and were more often APOE e4 positive than individuals with SCD in community-based cohorts and controls. Adjusted for random center effects, MMSE scores were lower in controls than in individuals with SCD. For all variables, individuals with SCD from the community were intermediate between SCD from a memory clinic and controls. Center characteristics are provided in Supplementary table A.

### Progression to AD and non-AD dementia

During follow-up 3.9±2.2 years (range 0.9-12.8 years), 84 (6% of 1391) controls without SCD progressed to dementia, of which 61 (66% of demented) to AD and 23 (33% of demented) and non-AD. Amongst individuals with SCD, 194 (7% of 2978) progressed to dementia, attributed to AD for 127 (65% of demented) or another type of dementia for 67 (35%). Within the non-AD dementias 30 (16% of all dementia cases) individuals with SCD progressed to vascular dementia, 8 (4%) progressed to FTLD, 9 (5%) to DLB, and 20 (10%) to another type of non-AD dementia. Figure 2 shows the percentages of dementia diagnoses in community cohorts and memory clinics. In a multilevel model, we compared percentages of dementia diagnoses in community and memory clinic cohorts, and found that individuals with SCD in community based cohorts more often received a diagnosis of vascular dementia (23% community vs. 9% memory clinic (p=0.01)). By contrast, diagnoses of DLB and FTLD were more frequently made in a memory clinic setting (DLB 8% memory clinic vs. 1% community (p=0.070); FTLD: 8% memory clinic vs. 0% community, model did not converge). The percentage of a diagnosis of AD did not differ between recruitment settings (67% vs. 63%, p=0.55), nor did the number of cases with 'dementia other' (memory clinic 8% vs. community cohort 13% (p=0.34)).

### Incidence rate of dementia

Among individuals with SCD, incidence rate of dementia was 17.7 (95% Poisson Confidence Interval 15.2-20.3) per 1000 person years. The incidence rate per 1000 person years for dementia due to AD was 11.5 (9.6-13.7) and for non-AD dementia 6.1 (4.7-7.7). In controls without SCD the incidence rate of dementia was 14.2 (11.3-17.6); 10.1 (7.7-13.0) for AD and 4.1 (2.6-6.0) for non-AD. Table 3 shows the incidence rates of dementia in memory clinics (20.0 (16.4-24.1)) and community cohorts (15.4 (12.3-19.0)) per decade, and for AD and non-AD dementia separately. Incidence rates increased with age, as visualized in figure 3 and 4. Multivariate Cox proportional hazards models showed that compared to controls without SCD, individuals with SCD in memory clinic cohorts are at a clearly increased risk of dementia (table 4).

The increased risk of dementia in community based cohorts did not reach significance. In addition, higher age, lower MMSE and APOE e4 carrier status

were independent predictors of incident dementia. We evaluated center random effects for MMSE and education in the Cox proportional hazards models, which did not specifically alter results, concluding that findings were generalizable for MMSE and education.

Stratified for AD or non-AD outcomes, the increased risk of dementia in individuals with SCD was particularly attributable to incident non-AD dementia's. Lower MMSE increased the risk of both AD and non-AD dementia (table 4), while higher age was associated with an increased risk of non-AD dementia. There was no significant effect of sex, education or APOE in the stratified analyses.
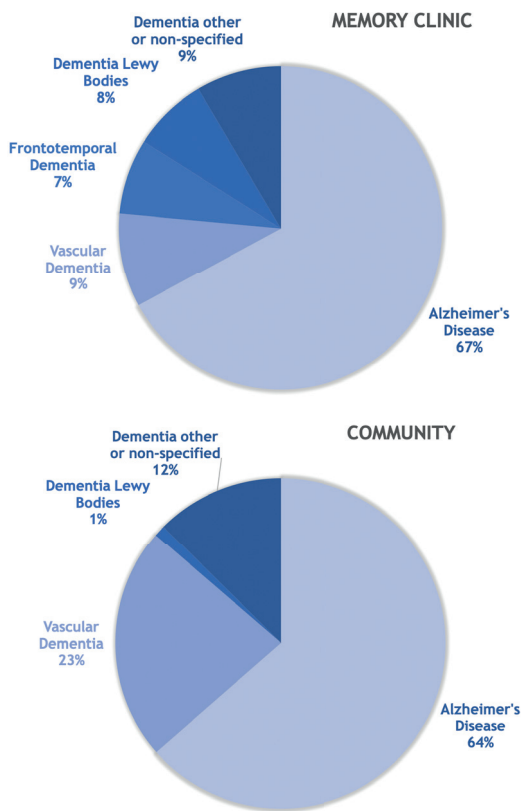


Figure 2. Type of dementia diagnosis in memory clinic and community settings. Total number of dementia diagnoses: memory clinic n=107, community n=87. Results are displayed as N (%).
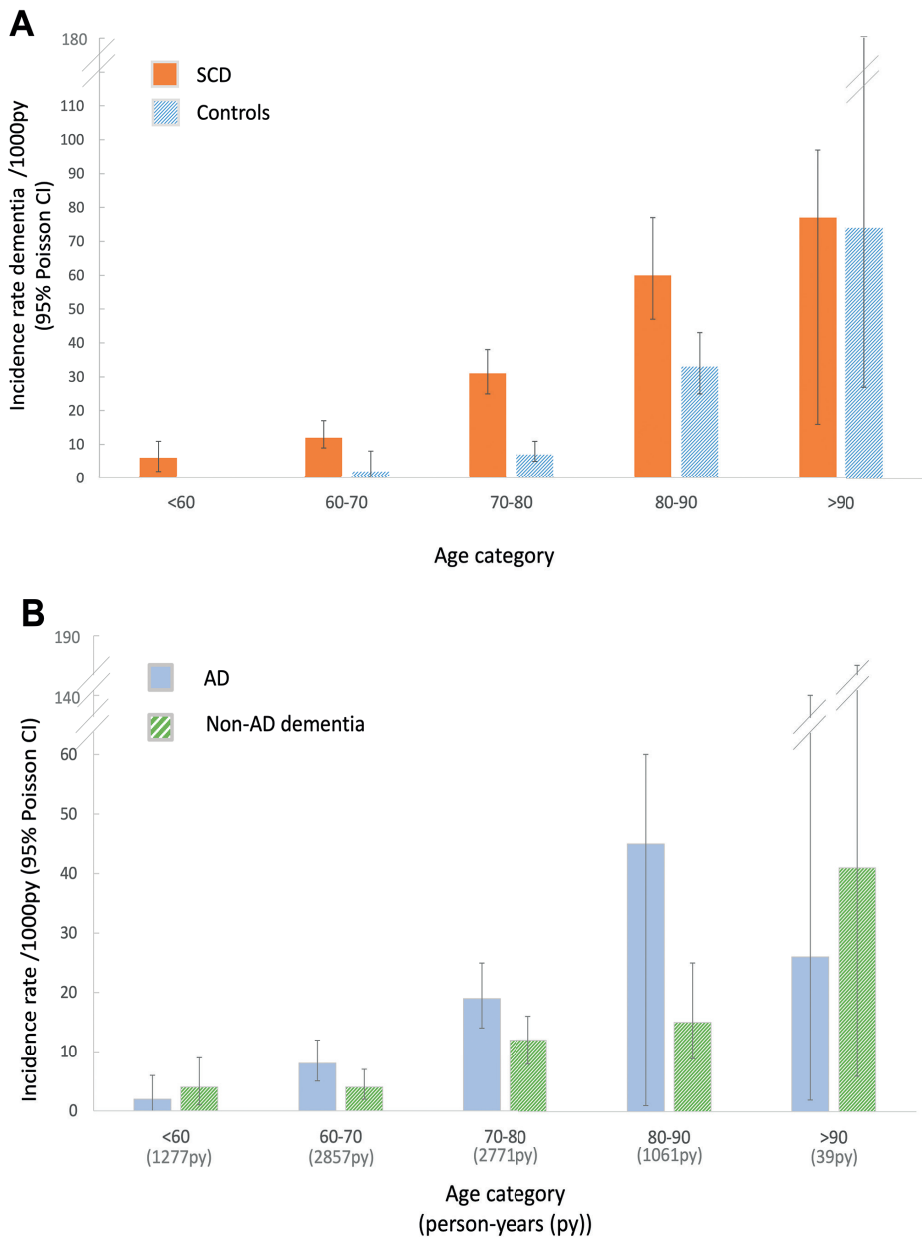
Figure **3A**: Incidence rates of dementia per decade in individuals with SCD and controls. Figure **3B**: Incidence rates of AD and non-AD dementia per decade. Results are presented as incidence rates per 1000 person-years (95% Poisson confidence intervals) per decade.
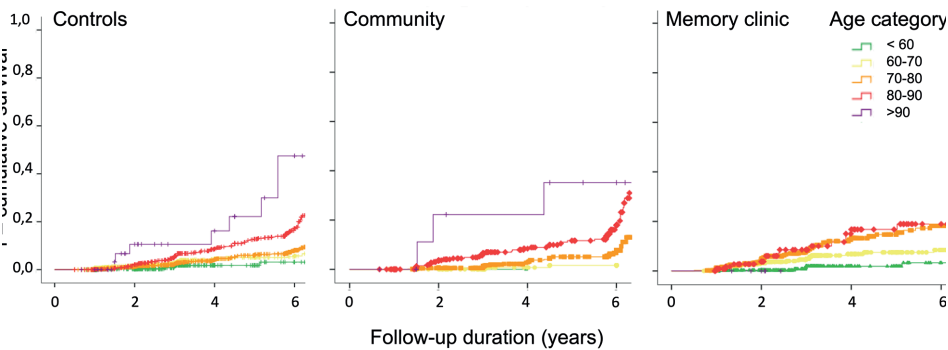
Figure 4. Kaplan Meier curves of the cumulative risk of progression to dementia per decade, stratified for recruitment setting.

## DISCUSSION

In this multi-center study including both memory clinic and community based cohorts of SCD, we evaluated incidence rates of dementia, both for AD and non-AD. We found an overall dementia incidence rate in individuals with SCD of 17.7 per 1000 person years, compared to 14.2 in controls without SCD. Particularly in memory clinic patients with SCD in a, the risk of non-AD dementia is strongly increased. In line with incidence studies of dementia subtypes [19,20], roughly one out of three incident dementia in individuals with SCD cases was due to non-AD. Of note, non-AD dementias in memory clinics often comprised FTD or DLB, while in community based cohorts, VaD was relatively more common. Other determinants of incident dementia included higher age, lower baseline cognition, and APOE e4 carriership.

Our data clearly showed that recruitment setting modifies the risk of progression from SCD to dementia. It is well known that recruitment setting (i.e. memory clinic vs community) affects studies in SCD [3,21,22]. However, the number of studies directly comparing recruitment setting is small [9,23–25]. A previous meta-analysis suggested that the annual conversion rate from SCD to dementia did not differ between memory clinics and community cohorts [9], and likewise at first sight, our memory clinic cohorts also had only slightly higher incidence rates (20.1/1000 person years) compared to community cohorts (15.4/1000 person years). However, heterogeneity between studies has repeatedly been mentioned in SCD studies [9,21]. And a recent study showed that progression to MCI is more common in individuals with SCD recruited at a memory clinic than in a community based setting [26]. We also observed great heterogeneity between cohorts in study design, center and patient characteristics, and to allow meaningful pooling of data, we used a multi-level statistical approach, carefully taking into account center differences. Particularly, memory clinic cohorts were on average a decade younger, explaining their overall lower incidence. When we stratified by age, our data revealed that in every age-bin, incidence of dementia is higher in the memory clinic than in the community based cohorts of individuals with SCD. The only exception was the oldest age-bin >90, where data in memory clinics were simply lacking and incidence of dementia in community based cohorts was high. Our data illustrate that memory clinic patients who actively seek help for their perceived cognitive problems, indeed, are more likely to experience the first (preclinical) signs of a neurodegenerative disease [23,24].

Our findings provide evidence that SCD is not only a potential harbinger of AD, but also of other dementias. Two third of incident dementias in individuals with SCD was attributable to AD dementia, whereas approximately one third was attributable to another type of dementia. The relative frequencies of individuals with SCD progressing to FTLD, DLB and VaD seemed comparable with previous dementia incidence studies [19,20,27–29]. In memory clinic cohorts, DLB and FTLD were more frequently diagnosed than in community cohorts. By contrast,

VaD was more often diagnosed in the older community cohort individuals with SCD. This difference could be a reflection of differing operationalization of diagnostic criteria for dementias, which may be handled differently between settings [30], e.g.: VaD in memory clinic settings often requires neuroimaging criteria, while in community based settings such a diagnosis may be based on clinical presentation only. Diagnosis of DLB or FTLD require careful neurological examination by an expert neurologist, available mostly in specialized clinics rather than community setting. Also, individuals with early VaD or DLB might be referred for evaluation to general neurology, instead of a memory clinic, as patients complain rather of neurological symptoms, such as parkinsonism or gait change, than memory decline. Furthermore, individuals with FTLD may be less likely to participate in voluntary studies, because of disease characteristics [31].

The large majority of individuals with SCD in both memory clinic and community-based cohorts did not progress to any type of dementia, but rather remained cognitively normal. Despite the growing interest in SCD as a putative first syndromatic stage of AD, the group of individuals seeking help where a neurodegenerative disorder can be excluded as cause of their problems, also merits our attention. From studies in the field of MCI and early studies in SCD, it is clear that e.g. CSF biomarkers have particularly good negative predictive value, illustrating that their optimal clinical use is for reassurance of individuals with normal biomarkers [32,33]. Alternative causes of SCD could be subclinical psychiatric disorders, personality traits or surmenage. Individuals with SCD unlikely to progress to AD or non-AD dementia could be reassured, and might benefit from counseling and/or lifestyle interventions, aiming to promote a healthy brain.

We evaluated which determinants contributed to an increased risk of progression from SCD to dementia, and found that higher age, lower baseline MMSE, APOE e4 status and recruitment setting resulted in an increased risk of dementia, which is consistent with the literature [9,34,35]. We found that higher age contributed relatively more to the risk of AD than non-AD dementias in individuals with SCD. A possible explanation could lie in the fact that some non-AD dementias, such as FTLD, are relatively more often diagnosed at a younger age, thus reflecting less contribution of a higher chronologic age in the risk of non-AD dementia in comparison with AD dementia [31]. The effect of MMSE on the risk of clinical progression also seemed stronger for AD than for non-AD. The MMSE is mainly designed as a global cognitive screening tool, and most sensitive for disturbances in memory and orientation [18]. Since the memory domain is relatively less affected in non-AD dementias, it is conceivable that  MMSE is less sensitive for non-AD dementias [36]. APOE e4 status was associated with an increased risk of dementia, which appeared to be attributable to the risk of AD, but not non-AD, which is in agreement with the literature [37,38].

Limitations of the study include the substantial heterogeneity in cohort

characteristics. The heterogeneity includes differences in demographics of participants between centers and substantial inherent center characteristics such as the definition of SCD, the administered SCD-questionnaires, the use of (MRI, PET or CSF) biomarkers in the diagnostic process, and the outcome measures (differences in dementia criteria used). Furthermore, recruitment setting has been shown to be a moderator of SCD results, as discussed above. Nonetheless, we were able to combine these cohorts by using a multilevel statistical approach, using shared-frailty Cox models and taking into account random center effects. Our results underline the importance of the harmonized research criteria for SCD, which have been put forward by the SCD-I working group [3,39]. In this study we had no information available on different domains of cognitive complaints, such as memory domain vs. non-memory domains. Since one third of dementia diagnoses were non-AD, evaluation of SCD in non-memory domains using questionnaires and also qualitative assessment is of interest to better understand the underlying pathology of SCD [40]. Also, we had no comprehensive cognitive test battery or biomarker data available for a large part of our cohort, and we cannot exclude the possibility of misdiagnosis in a number of cases. Future studies should include a wider range of cognitive tests and biomarkers to further evaluate the process of differentiating between AD and non-AD dementias. We did not take into account all available SCD cohorts, but this collaborative study did originate from ISTAART's SCD PIA, including all centers that wanted to contribute data. Strengths of the study, therefore, include the large sample of SCD patients, with participating centers from around the world. Furthermore, this is the first time that the incidence of non-AD dementia is evaluated in the context of SCD.

Members of the international SCD Working Group (SCD-I) have published a conceptual framework on SCD research to facilitate harmonization of SCD research [3,39]. The framework, however, is focused on the detection of preclinical AD, and not so much preclinical stages of other dementias. The risk of preclinical AD has been suggested to be specifically modified by self-reported memory decline [7], and a large overview of SCD measures indicated that most instruments indeed evaluate memory-specific decline [3,21]. However, since approximately one third of progressing patients in our SCD sample progressed to another type of dementia than AD, the importance of non-memory characteristics needs to be considered when evaluating SCD.

Table 1. Overview of participating centers and their characteristics

| Center | | Setting | Definition of SCD | Follow-up information | N total | N SCD | N controls |
|---|---|---|---|---|---|---|---|
| Alzheimer Disease Neuroimaging Initiative (ADNI) and Indiana Alzheimer Disease Center [41,42] | USA | Memory clinic <br> Multi-center with standardized methods | Normal cognition + MC visit because of complaints (assessed by CCI) | Annual FU | 372 | 126 | 246 |
| Australian Imaging, Biomarkers and Lifestyle Study (AIBL) [43] | Australia | Community <br> Multi-center with standardized methods | Normal cognition + Cognitive complaints question (Mac-Q): YES | FU interval 18 months | 1636 | 491 | 161 |
| Amsterdam Dementia Cohort [44-46] | The Netherlands | Memory clinic <br> Single-center | Normal cognition+ MC visit because of complaints | Annual FU | 463 | 463 | 0 |
| Barcelona Hospital Clinic i Universitari | Spain | Memory clinic <br> Single-center | Normal cognition+ MC visit because of complaints | Annual FU | 75 | 52 | 23 |
| German Dementia Competence Network (DCN) [47] | Germany | Memory clinic <br> Multiple single center studies | Normal cognition+ MC visit because of complaints <br> Participant did not meet Jak-Bondi criteria [48] | Annual FU | 256 | 256 | 0 |

| Study | Country | Setting | Definition | Follow-up | | | |
|---|---|---|---|---|---|---|---|
| Development of Screening Guidelines and Clinical Criteria for Pre-dementia AD study (DESCRIPA) [49] | Europe^ | Memory clinic / Multiple single center studies | Normal cognition+ MC visit because of complaints | Annual FU | 245 | 224 | 0 |
| Hellenic Longitudinal Investigation of Aging and Diet (HELIAD) [50] | Greece | Community / Single-center | Normal cognition + Cognitive complaints question (Mac-Q): YES | Annual FU | 531 | 154 | 309 |
| INSIGHT pre-AD study / Paris [51,52] | France | Community / Single-center | Normal cognition + Cognitive complaints question (Mac-Q): YES | FU after 3yr | 318 | 318 | 0 |
| Leipzig Longitudinal Study of the Aged (LEILA75+) [53] | Germany | Community / Single-center | Normal cognition + Cognitive complaints question (Mac-Q): YES | FU interval 18 months | 670 | 169 | 501 |
| Sydney Memory and Ageing Study (MAS) [54] | Australia | Community / Single-center | Normal cognition + Cognitive complaints question (Mac-Q): YES | FU interval 24 months | 467 | 316 | 151 |
| New York University Langone Medical Center (NYU) | USA | Memory clinic / Single-center | Normal cognition+ MC visit because of complaints | Annual FU | 488 | 409 | 0 |

Of the participating cohorts there were eight single center studies, two multi-center studies with standardized methods (ADNI and AIBL), and two cohorts composed of data from multiple single center studies (DCN Germany and DESCRIPA). ADNI and the single site Indiana ADC cohort were combined as both were assessed and included based on the Cognitive Change Index (CCI). ^To prevent possible overlap of participants, all cases from the VU medical center included in the DESCRIPA dataset were not included in analyses (n=22). MC = memory clinic; FU = follow-up. See supplementary table for more information.

**Table 2.** Baseline demographic features of study participants (N=4369)

|  | All | Controls | Community | Memory clinic | p-value |
|---|---|---|---|---|---|
|  | N=4369 | N=1391 | N=1448 | N=1530 |  |
| Number of cohorts |  | 6 | 5 | 6 |  |
| Age, year | 73±9 | 77±7 | 76±6 | 67±9 | 0.000 |
| Female sex | 2611 (60%) | 889 (64%) | 901 (62%) | 821 (54%) | 0.000 |
| MMSE | 28.3±1.7 | 27.9±1.9 | 28.4±1.4 | 28.4±1.6 | 0.000[a] |
| Education, year^ | 12±4 | 11±5 | 10±4 | 14±4 | 0.000 |
| APOE e4 carrier# | 888 (28%) | 184 (26%) | 261 (22%) | 443 (36%) | 0.000 |
| Follow up, year | 3.9±2.2 | 4.3±2.0 | 3.9±2.0 | 3.5±2.3 | 0.000 |

Values displayed as unadjusted mean ± standard deviation (SD) or N (%). Differences between memory clinic and community cohorts were assessed using linear mixed models (continuous variables) or generalized estimating equations (dichotomous variables) taking into account center random effects. a When taking into account center random-effects, MMSE is lower in controls vs. community cohorts vs. memory clinic cohorts (p<0.001), whereas unadjusted means are shown in the table. ^ data available for 77% (memory clinic 98%, community 44%, controls 88%), # data available for 72% (availability memory clinic 82%, community 81%, controls 51%).

**Table 3.** Incidence rates of dementia, and dementia due to AD and non-AD

| | | | Incidence rate / 1000 person years (95% Poisson confidence intervals) | | |
|---|---|---|---|---|---|
| | | Number of person years | Dementia | AD | Non-AD |
| Controls (n=1391) | All | | 14.2 (11.3-17.6) | 10.1 (7.7-13.0) | 4.1 (2.6-6.0) |
| | Age category | | | | |
| | <60 | 55 | 0 | 0 | 0 |
| | 60-70 | 859 | 2 (0-8) | 2 (0-8) | 0 |
| | 70-80 | 3379 | 7 (5-11) | 4 (2-7) | 3 (2-6) |
| | 80-90 | 1547 | 33 (25-43) | 25 (18-34) | 8 (4-14) |
| | >90 | 81 | 74 (27-161) | 62 (20-144) | 12 (0-69) |
| Community (n=1448) | All | 5647 | 15.4 (12.3-19.0) | 9.7 (7.3-12.7) | 5.7 (3.9-8.0) |
| | Age category | | | | |
| | <60 | 12 | 0 | 0 | 0 |
| | 60-70 | 904 | 2 (0-8) | 2 (0-8) | 0 |
| | 70-80 | 3222 | 11 (8-15) | 6 (4-9) | 5 (3-8) |
| | 80-90 | 1509 | 32 (24-42) | 23 (16-32) | 9 (5-16) |
| | >90 | 47 | 60 (12-175) | 20 (1-111) | 40 (5-145) |
| Memory clinic (n=1530) | All | 5355 | 20.1 (16.4-24.1) | 13.4 (10.5-16.9) | 6.5 (4.6-9.1) |
| | Age category | | | | |
| | <60 | 1271 | 6 (2-11) | 2 (0-6) | 4 (1-9) |
| | 60-70 | 1952 | 17 (12-24) | 11 (7-16) | 6 (3-11) |
| | 70-80 | 1571 | 32 (24-42) | 22 (15-30) | 10 (6-16) |
| | 80-90 | 447 | 26 (20-58) | 31 (17-52) | 4 (1-15) |
| | >90 | 12 | 0 | 0 | 0 |

Results are displayed as incidence rates (95% Poisson Confidence Interval) per 1000 person-years.
Analyses stratified for AD and non-AD dementias, age category and recruitment setting.

**Table 4.** Associations between determinants and the risk of dementia in general, and dementia due to AD and non-AD in a combined SCD sample (n=4369)

| Predictor | Dementia | | AD | | Non-AD | |
|---|---|---|---|---|---|---|
| | Model 1 | Model 2 | Model 1 | Model 2 | Model 1 | Model 2 |
| **Controls** | ref | ref | ref | ref | ref | ref |
| **SCD community** | 2.1 (0.6 – 7.7) | 1.5 ( 0.4 – 5.8) | 1.8 (0.8 – 4.1) | 0.7 (0.3 – 1.9) | 4.6 (1.1-19.0) | 2.2 (0.5-9.7) |
| **SCD memory clinic** | 10.0 (2.9 – 34.0) | 10.4 (3.4 – 32.0) | 1.7 (0.8 – 3.6) | 2.0 (1.0 – 4.1) | 12.7 (3.1-51.4) | 7.1 (1.8-27.3) |
| Age, years | 1.1 (1.1-1.1) | 1.1 (1.1-1.1) | 1.0 (1.0-1.0) | 1.0 (1.0-1.0) | 1.09 (1.05-1.12) | 1.07 (1.02-1.11) |
| Female sex | 1.1 (0.9-1.4) | 1.0 (0.7-1.5) | 1.0 (0.9-1.04) | 1.0 (0.9-1.1) | 0.8 (0.5-1.3) | 0.6 (0.3-1.0) |
| MMSE [h] | | 0.7 (0.65 – 0.8) | | 0.95 (0.92-0.98) | | 0.8 (0.7-0.9) |
| Education, years^ | | 1.0 (0.97-1.1) | | 1.0 (0.98-1.01) | | 1.0 (0.9-1.1) |
| APOE e4 status# | | 1.8 (1.3-2.5) | | 1.0 (0.9-1.1) | | 1.1 (0.6-2.1) |

Results are presented as hazard ratio's (95% confidence interval) and reflect the risk of progression from SCD to dementia in general, and dementia due to AD and non-AD. **Model 1**: age and gender adjusted. **Model 2**: additionally adjusted for MMSE, education in years and APOE e4 status (dus to missing data in MMSE, education and APOE model 2 has less observations). HR's were calculated per determinant in univariate models and combined in multivariate models. ^ data available for 77%, # data available for 72%. h=higher scores reflect better performance.

**SUPPLEMENT TABLE.** Demographic features per cohort

| Center | Normal cognition at baseline | N | Age, years | Gender, F(%) | MMSE | Education, years ^ | Follow-up duration, years | APOE e4 carrier # |
|---|---|---|---|---|---|---|---|---|
| ADNI and Indiana ADC | SCD | 126 | 70±8 | 77 (61) | 28.9±1.3 | 17±2 | 2.9±2.2 | 47 (38) |
| | Controls | 246 | 72±7 | 138 (56) | 28.5±1.8 | 17±3 | 3.2±1.9 | 81 (33) |
| AIBL Australia | SCD | 491 | 74±7 | 295 (60) | 28.7±1.3 | n/a | 4.3±2.0 | 123 (25) |
| | Controls | 161 | 73±7 | 95 (59) | 28.9±1.2 | n/a | 5.8±0.7 | 52 (32) |
| ADC Amsterdam | SCD | 463 | 62±10 | 212 (46) | 28.4±1.6 | 12±3 | 3.1±2.3 | 158 (39) |
| | Controls | n/a | - | - | - | - | - | - |
| Barcelona | SCD | 52 | 65±7 | 13 (25) | 28.1±1.7 | 11±4 | 3.5±1.6 | 13 (26) |
| | Controls | 23 | 66±9 | 12 (52) | 28.7±1.0 | 11±5 | 5.4±2.7 | 3 (13) |
| DCN Germany | SCD | 256 | 66±8 | 97 (38) | 28.0±1.7 | 13±3 | 2.2±0.9 | 86 (40) |
| | Controls | n/a | - | - | - | - | - | - |
| DESCRIPA Europe | SCD | 224 | 68±8 | 116 (52) | 28.4±1.5 | 11±4 | 2.5±0.8 | 53 (41) |
| | Controls | n/a | - | - | - | - | - | - |
| HELIAD Athens | SCD | 154 | 73±6 | 104 (68) | 27.7±2.2 | 6±3 | 2.9±0.7 | n/a |
| | Controls | 267 | 75±6 | 188 (61) | 27.0±2.6 | 6±5 | 2.8±0.6 | n/a |
| INSIGHT Paris | SCD | 318 | 76±3 | 204 (64) | 28.7±1.0 | n/a | 1.9±0.2 | 58 (18) |
| | Controls | n/a | - | - | - | - | - | - |
| LEILA Leipzig | SCD | 169 | 82±5 | 119 (70) | 27.5±1.6 | 12±2 | 4.9±2.2 | 10 (19) |
| | Controls | 501 | 81±5 | 368 (74) | 27.6±1.1 | 12±2 | 4..9±2.2 | 21 (15) |
| MAS Sydney | SCD | 316 | 78±5 | 179 (57) | 28.5±1.2 | 12±4 | 5.2±1.4 | 70 (23) |
| | Controls | 151 | 78±5 | 88 (58) | 28.5±1.1 | 11±3 | 5.1±1.5 | 27 (18) |
| NYU ADC New York | SCD | 409 | 70±10 | 306 (75) | 28.7±1.6 | 16±2 | 5.3±2.7 | 85 (26) |
| | Controls | n/a | - | - | - | - | - | - |

n/a = not available. ^ data available for 77%, # data available for 72%.

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# CHAPTER 4
## Personalized risk for clinical progression in cognitively normal subjects - the ABIDE project
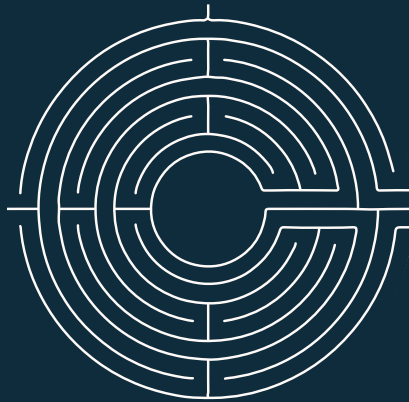
Van Maurik I.S., **Slot R.E.R.**, Verfaillie S.C.J., Zwan M.D., Bouwman F.H., Prins N.D.,
Teunissen C.E., Scheltens P., Barkhof F., Wattjes M.P., Molinuevo J.L., Rami L.,
Wolfsgruber S., Peters O., Jessen F., Berkhof J., Van der Flier W.M.;
Alzheimer's Disease Neuroimaging Initiative.

## ABSTRACT

**Introduction**: Biomarkers such as cerebrospinal fluid (CSF) and magnetic resonance imaging (MRI) have predictive value for progression to dementia in patients with mild cognitive impairment (MCI). The pre-dementia stage takes far longer and the interpretation of biomarker findings is particular relevant for individuals who present at a memory clinic, but are deemed cognitively normal. The objective of the current study is to construct biomarker based prognostic models for personalized risk of clinical progression in cognitively normal individuals presenting at a memory clinic.

**Methods**: We included 481 individuals with subjective cognitive decline (SCD) from the Amsterdam Dementia Cohort. Prognostic models were developed by Cox regression with patient characteristics, MRI and/or CSF biomarkers to predict clinical progression to MCI or dementia. We estimated five and three-year individualized risks based on patient-specific values. External validation was performed on ADNI and an European dataset.

**Results**: Based on demographics only (Harrell's C=0.70), five and three-year progression risks varied from 6%[3-11] and 4%[2-8] (age 55, MMSE 30) to 38%[29-49] and 28%[21-37] (age 70, MMSE 27). Normal CSF biomarkers strongly decreased progression probabilities (Harrell's C=0.82). By contrast, abnormal CSF markedly increased risk (five-year: 96%[56-100], three-year: 89%[44-99]). The CSF model could reclassify 58% of the individuals with an 'intermediate' risk (35-65%) based on the demographic model. MRI measures were not retained in the models.

**Discussion**: The current study takes the first steps in a personalized approach for cognitively normal individuals, by providing biomarker-based prognostic models.

## BACKGROUND

Dementia disorders place a huge burden on society and are set to bulge due to an aging population.[1, 2] Alzheimer's Disease (AD) is the most common cause of dementia and represents the largest unmet medical need in neurology.[3] In order to bring therapy and support to individuals as timely and accurate as possible, diagnostic tests play a key role. In individual patients with mild cognitive impairment (MCI),  biomarkers such as cerebrospinal fluid (CSF) and magnetic resonance imaging (MRI) have been shown to have predictive value for progression to dementia.[4] However, the pre-dementia stage of AD takes far longer, as neuropathological changes already start in the cognitively normal stage.[3, 5-8] For that reason, recent criteria proposed a biological framework for AD in which AD is classified based on the presence of  pathology rather than the presence of clinical symptoms.[8]

On a group level, AD biomarkers are predictive in cognitively normal individuals as well. For example, a reduced hippocampal volume on MRI been associated with an increased risk of clinical progression.[9-11] Furthermore, an abnormal AD biomarker profile in CSF has been shown to be strongly associated with clinical progression.[12-16] Moreover, even relatively low amyloid ß1-42 (Aß) levels, yet within the normal range, have been associated with clinical progression, indicating that simple dichotomous cut-offs fail to extract all information available in these markers and moreover, may erroneously reassure individuals.[17]

The question how these findings on group level translate to the individual is particular relevant for individuals who present with worries about their memory at a memory clinic, but are deemed cognitively normal. Unfortunately, findings on group level cannot be translated directly to the individual and the interpretation of biomarkers is not optimized. In addition, the meaning of biomarkers should ideally be interpreted in the context of an individual's own characteristics[18], but information on how to weigh and combine multiple sources of information is lacking. Therefore, clinicians are generally reluctant to disclose biomarker results to cognitively normal individuals. Nonetheless, individuals and caregivers, become increasingly assertive, demanding more specific, and individually tailored information.[19]

The objective of this study was to optimize the interpretation of biomarkers by composing individualized prediction models for clinical progression to MCI or dementia based on MRI and/or CSF biomarkers, that could be used in cognitively normal individuals.

## METHODS

### Participants
We included 481 cognitively normal individuals from the Amsterdam Dementia Cohort (ADC) and ongoing SCIENCe project, with a baseline diagnosis of subjective cognitive decline (SCD), available baseline MMSE and available baseline MRI and/or CSF data.[20, 21] All individuals had their baseline visit in our memory clinic between January 2000 and November 2015. Individuals with a diagnosis of MCI or dementia within 6 months after baseline were excluded from the analysis as they were likely to have been misclassified at baseline. Baseline diagnostic work-up consisted of a standardized one-day dementia screening.[20, 21] Clinical diagnosis was made by consensus in a multi-disciplinary meeting. Individuals were labeled with SCD if they presented with cognitive complaints, had normal results on clinical assessments and did not meet criteria for MCI, dementia or any other neurologic or psychiatric disorder known to cause cognitive complaints (i.e. cognitively normal).[22] Standardized annual follow-up included a follow-up visit with the neurologist and neuropsychologist and diagnoses were re-evaluated in a multi-disciplinary meeting.[20] Until early 2012, MCI was diagnosed according to Petersen's criteria and from 2012 onwards the diagnosis of MCI was based on National Institute on Aging-Alzheimer's Association (NIA-AA) criteria.[23, 24] The diagnosis of AD-dementia and other types of dementia were based on international diagnostic or research consensus criteria.[25-28]
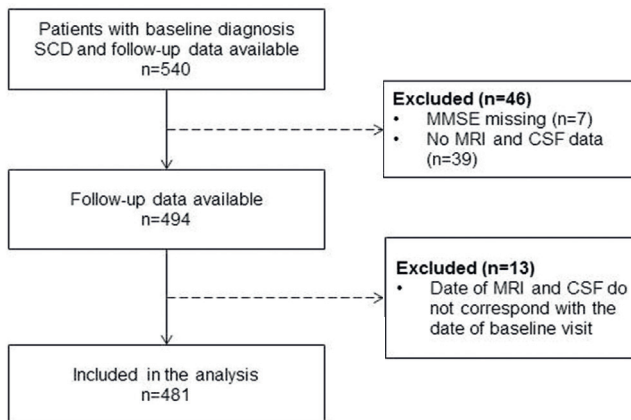


Supplementary figure 1. Inclusion of study participants

### MRI
Before 2008, brain MRI was performed on 1.0 and 1.5T MRI systems (Siemens Magnetom Avanto, Vision, Impact and Sonata, GE Healthcare Signa HDXT). From 2008 on, MRI of the brain was performed on 3T MRI systems (MR750, GE Medical Systems, Ingenuity TF PET/MR, Philips Medical Systems; Titan, Toshiba Medical

Systems ). The standard dementia protocol with whole brain coverage included near-isotropic sagittal 3D T1-weighted images (including oblique coronal reconstructions), sagittal 3D T2-weighted fluid-attenuated inversion recovery (FLAIR) (including axial reconstructions), axial T2-weighted turbo spin-echo and axial T2*-weighted gradient echo sequence or alternatively SWI sequences. MRI data was available for 432 (90%) individuals. Bilateral hippocampal volume (HCV, mL) was estimated using FMRIBs Integrated registration and segmentation tool (FIRST).29 Normalized brain volumes (NWBV, mL) were estimated with SIENAX (Structural Image Evaluation using Normalization of Atrophy Cross-sectional) using optimized settings.30 Additionally, visual rating of MRI was performed according to semi-quantitative visual rating scales for medial temporal lobe atrophy (MTA, 0-4) and global cortical atrophy (GCA, 0-3).31, 32

**CSF analyses**
CSF was obtained by lumbar puncture and collected in polypropylene tubes (Sarstedt, Nurmberg, Germany) and processed according to international guidelines.33 CSF biomarkers Amyloid ß1-42 (Aß ) and total Tau (tau) were measured using sandwich Enzyme-Linked Immuno Sorbent Assay (ELISA) on a routine basis (Innotest, Fujirebio, Gent, Belgium).34 Baseline CSF data was available for 344 (72%) individuals.

**Statistical analysis**
All analyses were carried out in STATA 14SE. Prognostic models were constructed with Cox regression analysis (determinants as continuous measures; CSF biomarkers log-transformed). The models were constructed with complete cases only and therefore the number of individuals varied across models. No differences in demographic characteristic or baseline survival were found between individuals with complete data and incomplete data (eTable 1). The clinical end-point was MCI or dementia.23-28
First, a prognostic model based on patient characteristics (age, gender and MMSE) and interactions between the characteristics was constructed. Subsequently, we added either MRI biomarkers (volumetric measures: HCV, NWBV or visual ratings: MTA, GCA ), CSF biomarkers (Aß , Tau) or both to the model. The models with volumetric MRI measures were adjusted for field strength. In all analyses, we intensively investigated main effects of patient characteristics and interaction effects between biomarkers and between biomarker and patient characteristics. Effects were retained in the model via a backward selection procedure, if p-value≤.10. The prognostic accuracy of the model was measured by Harrell's C-statistic.
We estimated cumulative progression probabilities with 95 percent confidence intervals using the survci command in STATA.35 We report five, three and one year cumulative progression probabilities with corresponding confidence intervals. Since the clinical follow-up visit times showed some variation, the cut-off for one year follow-up was set at 1.5 years, for three-year follow-up at 3.5 years and for five-year follow-up at 5.5 years. As an example, we provide risk estimates for individuals with an age of 55 and 70, females and males and

MMSE scores of 30 or 27. To contrast individuals with normal and abnormal MRI and CSF results, we entered 10th and 90th percentile MRI and CSF values in the Cox model. Note that when using the models, any value can be entered for a variable. Based on the constructed models, 5-year progression probabilities were calculated for every patient in the cohort. Subsequently we labeled each individual as having low risk ($\leq$ 35%), intermediate risk (35-65%) or high risk (>65%).In an additional set of analyses, we repeated all analyses to construct models predicting progression to MCI or AD-dementia as clinical end-point. In this set of analyses, individuals progressing to non-AD were censored at time of diagnosis of non-AD dementia.

*Validation*
We internally validated the models by five-fold cross validation, in which we again applied a backward selection procedure. Next, we performed external validation of our models on a sample comprising individuals with SCD from Alzheimer's Disease Neuroimaging Initiative (ADNI; n=92), Dementia Competence Network (DCN; n=86) and Barcelona Memory Clinic (n=41). Like the ADC cohort, DCN and Barcelona included individuals that went to the memory clinic to seek medical help and were labeled with SCD when cognitive testing could not confirm their cognitive complaints and criteria for MCI, dementia or other neurological or psychological diseases were not met. ADNI on the other hand is a population-based study. Subjects were labeled with SCD when a significant subjective memory concern was reported by the subject, informant or clinician. CSF was measured with Innotest in the DCN and Barcelona cohort and with Elecsys in ADNI. Therefore, biomarker values were standardized for the analysis to remove measurement levels. Patient characteristics from the cohorts can be found in eTable 2. Differences between the cohorts included a higher age in the ADNI cohort, higher progression rates in the DCN, longer follow-up for ADC and Barcelona individuals and ADNI and Barcelona individuals were more often female (eTable 2). Established models were fitted to the validation data and Harrell's C statistics were calculated.

**RESULTS**

During a mean follow-up of 3±2 years, 70(15%) individuals showed clinical progression to MCI (n=49), AD-dementia (n=10) or non-AD dementia (n=11). Mean age was 62±9 years, 211 (44%) of the individuals were female and the mean MMSE score was 28±1.6 (Table 1).
Table 2 shows the variables and corresponding coefficients included in the models (demographics only, CSF model, MRI volumetric model and MRI visual model).

The demographics only model included age and MMSE (sex not included, p-value>.10). Harrell's c-statistic was 0.70 (Table 2). Younger individuals, (as an example we set age at 55), with MMSE-scores of 30 had a low risk of progression: after five-years 6%[3-11], after three-years 4%[2-8] and after one-year 2%[0-2]. On the other end of the spectrum, older individuals (70) with lower MMSE-scores (27) had higher progression probabilities; risk of progression after five-year was 38%[29-49], after three-year 28%[21-37] and after one-year 11%[7-16] (Table 3). When we evaluated MRI markers, neither volumetric nor visual measures added predictive value over the demographic model including age and MMSE (p-value>.10). In the CSF model, female gender, higher age, lower MMSE score, lower Aß and higher Tau values were predictive of progression. Moreover, an interaction between Tau and age retained in the model. Tau was more predictive than Aß, especially in younger individuals (Tau*age p-value<.01, Table 3 and Figure 1). Harrell's C-statistic was 0.82 (Table 2, similar when p-tau was included instead of tau (eTable 3). To contrast individuals with normal and abnormal CSF results, we derived probabilities for individuals with 10th and 90th percentile CSF values from the model. Abnormal Aß and Tau resulted in high five, three and one year progression risks; 96% [56-100], 89% [44-99] and 51% [18-92]. By contrast, normal CSF biomarkers strongly decreased progression probabilities to 1%[0-3] in five year, 0%[0-2] in three year and 0%[0-1] in one year, indicating the negative predictive value of these biomarkers. Please note that we report examples, as the model provides risks for any given value.

Figure 2 shows the distribution of five-year progression probabilities based on the model including CSF biomarkers. The majority of individuals, 84% (n=290) were labeled as having low risk of progression, 12% (n=41) had intermediate risk of progression, and 4% (n=13) had high risk of progression. Of note, 58% of the individuals that were classified as 'intermediate' based on the demographic model, could be reclassified as having low (49%) or high (9%) risk according to the CSF model (eTable 4).

In an additional set of analyses, we repeated the analysis restricting the outcome to progression to MCI or AD dementia. The prognostic accuracy of the CSF model increased in line with specificity for AD of the biomarkers under evaluation. Harrell's C statistic remained 0.70 for the demographic model and increased to 0.84 for the CSF model.

Internal validation by fivefold cross validation confirmed prognostic performance in both models (eTable 5); cross validation of the demographic model resulted in Harrell's c statistics ranging from 0.63-0.77. The model with CSF biomarkers showed cross validated Harrell's C ranging from 0.75-0.90. External validation showed moderate performance of the models (demographic model: Harrell's C=0.62; CSF model: C= 0.68).
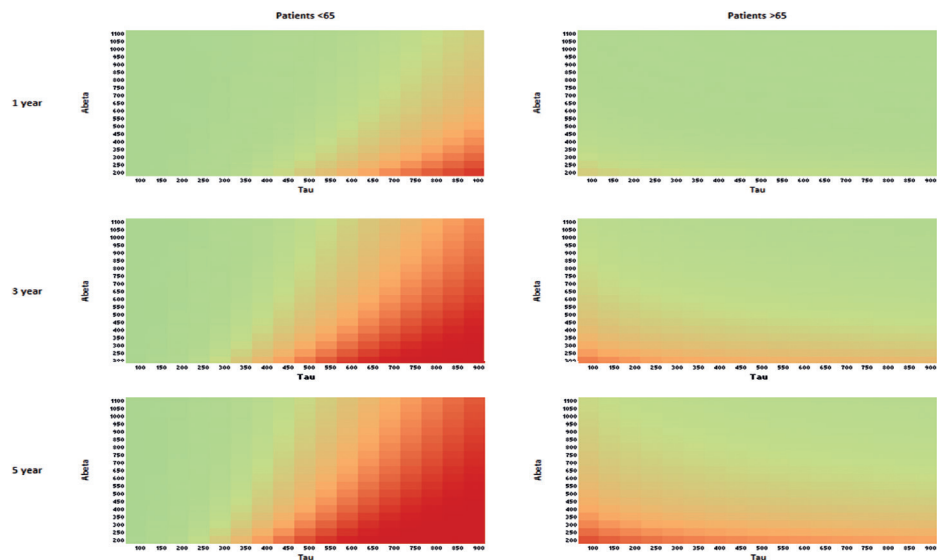


Figure 1. Probability of progression within one (upper panel) and three (middle panel) and five (lower panel) year based on Aβ (pg/mL; y-axis) and Tau (pg/mL; x-axis), stratified for individuals younger (left) and older than 65(right).
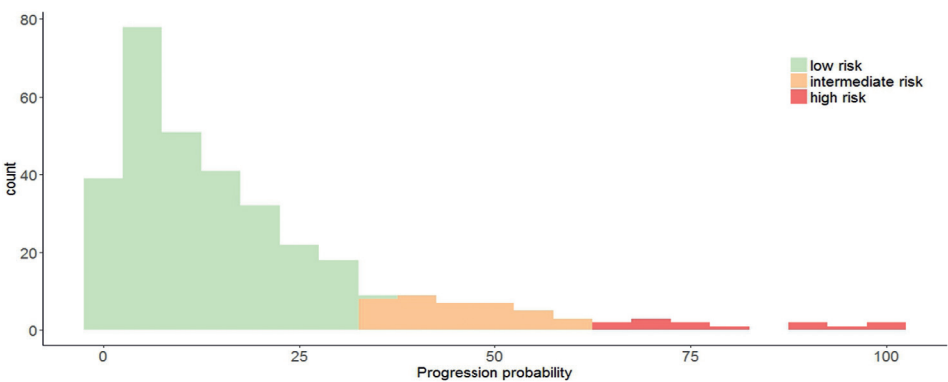


Figure 2. Distribution of five year progression probabilities based on the CSF model. Green: low risk 0-35%, orange: intermediate 35-65%, red: high risk 65-100%.

## DISCUSSION

In this study, we constructed biomarker prediction models that provide individual risk estimates of clinical progression in order to optimize the interpretation of biomarkers for cognitively normal SCD individuals. CSF biomarkers considerably improved prognostic performance over the use of age and MMSE only. This was mostly driven by their strong negative predictive value.

Alzheimer pathology as reflected in biomarker changes presumably starts more than twenty years before the onset of dementia.[3, 8] Clinicians are reluctant to disclose biomarker results to cognitively normal individuals presenting at a memory clinic, as former findings that were based on group level cannot directly be translated to the individual. Moreover, there is always a degree of uncertainty associated with the interpretation of biomarkers. With our models, we provide a first step towards a framework for a personalized approach, allowing the use of biomarker results for cognitively normal individuals presenting at memory clinics. This can be useful, as individuals and caregivers become increasingly assertive in their need for information on their risk of dementia. Moreover, interest in individualized risk profiling and both primary and secondary prevention strategies is increasing rapidly. Although truly longitudinal data are lacking, our sample allowed to infer predictions of progression over periods of three and even five years, which has great relevance for individuals and their family members. Probabilities of progression within one year in SCD individuals remain low, and this is in line with the notion that outcome at one-year follow-up is not a reasonable time frame for SCD, as these individuals initially perform cognitively normal.

Former studies have shown the clinical relevance of CSF biomarkers in pre-dementia individuals on a group level.[15, 16] In the current study, we found that Tau was a stronger predictor than Aß. Particularly, Tau was more predictive for progression in younger individuals. Abnormal Tau values in older individuals were less predictive, probably due to normal aging processes or multiple pathologies in older individuals.[36] Moreover, gender was included as a predictor in the CSF model, meaning that CSF measures should also be interpreted in the context of a patient's gender. This fits with findings from a recently published review that showed the importance of sex differences for patient stratification and personalized treatment.[37] With these CSF biomarkers and patient characteristics, 88% of the individuals could be classified as having a high (>65%) or low (≤ 35%) risk of clinical progression within 5 years.

Former studies on MRI biomarkers have reported that cognitively normal individuals with SCD had lower hippocampal volumes compared to healthy controls.[11] Moreover, hippocampal atrophy and lower brain volumes predicted of progression to MCI and/or dementia.[10, 38] However, these previously reported significant results for MRI were mostly based on small absolute differences between groups of individuals, precluding their usefulness in individualized risk predictions. In the current study, MRI markers did not improve personalized risk estimates over the use of age and MMSE only. The effects of MRI biomarkers

lost significance, as soon as age was included in the model, suggesting that the observed atrophy in this population is largely attributable to aging and/or did not capture additional predictive value over subtle cognitive impairment.

In a former study in MCI, we found that MRI biomarkers in combination with patient characteristics and also CSF biomarkers improve individualized prediction of progression to dementia.4 The finding that atrophy on MRI has less predictive value in cognitively normal individuals than in MCI patients, is consistent with the hypothetical biomarker model which suggests that CSF biomarker changes precede MRI-based estimates of neurodegeneration.39

Among the limitations, we found that the models showed somewhat less prognostic performance in external cohorts. This may be attributable to the fact that the outcome in the current study is clinical progression to MCI or dementia. While dementia is a relatively definitive end-point, MCI patients may still remain stable or convert to normal states of cognition and variability in this diagnosis between centers may be larger than in case of dementia.3, 24, 40 In addition, external validation is highly dependent on the case-mix of a sample. In the field of SCD, one of the most important unresolved challenges is the variability of defining SCD across studies.41-43 In the ADC, DCN and Barcelona cohort all individuals went to the memory clinic seeking help and their complaints might be more severe than in the population-based ADNI cohort. In ADNI, individuals were labeled with SCD when a significant subjective memory concern was reported by the subject, informant or clinician. Moreover, the standardized diagnostic work-up differed between the centers. For example, ADNI measured CSF with Elecsys instead of Innotest and brain volume with Freesurfer software instead of FSL FIRST. However, we limited these differences as much as possible by standardizing the biomarker values to remove measurement levels.

Another limitation is that we used different scanners and field strengths. This however, resembles real life clinical practice, and we included field strength as an additional determinant in the models. Field strength, however, did not improve predictive ability of MRI models. In addition, we used MMSE as a measure of global cognition in our models, which has been described as an insensitive instrument in preclinical stages. Other measures for cognition (for example a composite score of specific items of the MMSE and Clinical Dementia Rating scale; ADCOMS44, or instrumental activities of daily living (IADL45)) with higher sensitivity could improve the models. Such an approach might be subject for future studies. Lastly, the follow-up duration varied between individuals and the mean follow-up period of 3±2 years was rather short in comparison with the assumed duration of the stage of preclinical AD. Nonetheless, we had enough power to estimate risks over a period of 5 years, which is a considerable duration of follow up.

A major strength of this study is the simplicity of the models. Often, the goal of constructing prediction models is to derive the most optimal combination of many variables. However, such models often require multiple pieces of data that are not easily available, consequently limiting their clinical footprint.46 In the current paper, we took a different approach as we aimed to optimize

the interpretation of MRI-measures and CSF biomarkers in individuals with SCD: given that a clinician has decided to obtain MRI and/or CSF biomarkers in an individual with SCD with a given age, sex, MMSE, this clinician wants to make optimal use of the results of MRI and CSF. By doing so, our models helps to interpret biomarkers in the individual patient (hence; personalized) and shows proof of principle that personalized predictions could be feasible in very early stages of AD. Another strengths is that we used a large sample of SCD individuals. All individuals had an extensive screening at baseline to rule out MCI or other neurological causes of memory complaints and careful follow-up, with diagnosis re-evaluated in a multidisciplinary setting, which has contributed to the robustness of the data. The vast majority of individuals came to the memory clinic with worries about their cognitive functioning, rendering our models highly relevant for this population. In fact, this population is comparable to what in the new Alzheimer research framework is described as clinical stage 2.[8] Our results confirm clinical validity of such stage 2, as the presence of Alzheimer biomarkers strongly increases the risk of future progression to MCI or dementia. Another strong aspect of this study is that we accompany predictions with confidence intervals, which gives a good indication on precision of the prediction.

In an earlier study on communication of diagnostic test results, individuals and caregivers who recently visited a memory clinic indicated that they wanted more information on their prognosis and what test results meant for their personal lives ('what do these results mean for my future').[47] Nonetheless, clinicians tend to be reluctant to disclose biomarker results to cognitively normal individuals. The major concern is that disclosure could increase anxiety.[48] An argument against the disclosure of risk, is the lack of treatment options. But this raises the question whether it is ethical to withhold individuals from information that is actually available. Moreover, our models show that particularly the negative predictive value of the models is good, suggesting that biomarker results can be especially valuable to reassure patients.

## Conclusions

In conclusion, we constructed prognostic models that allow interpretation of biomarker data in cognitively normal individuals in a memory clinic at the individual level. In light of future disease-modifying drugs, risk prediction on an individual level becomes increasingly important.[49] By integrating biomarker results and demographic characteristics in AD risk modeling, the current study takes the first steps in a personalized approach for cognitively normal individuals.[48, 50] This is especially valuable for the reassurance of individuals with normal biomarkers, since clinical progression over a period of five years is very unlikely for them.

**Table 1.** Baseline characteristics

|  | SCD individuals (n=481) |
|---|---|
| No.(%)  with clinical progression | 70 (15%) |
|  Progression to MCI | 49 (10%) |
|  Progression to AD dementia | 10 (2%) |
|  Progression to non-AD dementia | 11 (2%) |
| Age | 62±9 |
| Gender, No.(%) females | 211 (44%) |
| MMSE | 28±1.6 |
| Follow-up duration | 3±2 |
| Medial Temporal Lobe Atrophy | 0.4±0.5 |
| Global Cortical Atrophy | 0.4±0.6 |
| Hippocampal Volume (cm3) | 7.2±1 |
| Normalized Whole Brain Volume (cm3) | 1453±100 |
| Amyloid ß1-42 | 879±260 |
| total tau | 298±196 |
| phosphorylated tau 181 | 49±22 |

Data are mean ± standard deviation, unless otherwise specified. MMSE= mini-mental state exami-nation, MRI=magnetic resonance imaging, CSF=cerebrospinal fluid.

**Table 2.** Regression coefficient of the final model

| | MCI/Dementia | | | | MCI/AD-dementia | | | |
|---|---|---|---|---|---|---|---|---|
| | Coëfficient | Standard Error | P-value | Harrell's C | Coëfficient | Standard Error | P-value | Harrell's C |
| **Demographic (n=481)** | | | | | | | | |
| Age | 0.0854 | 0.0147 | <.001 | 0.70 | 0.0904 | 0.0162 | <.001 | 0.70 |
| MMSE | -0.2497 | 0.0639 | <.001 | | -0.2236 | 0.0726 | <.01 | |
| **CSF (n=344)** | | | | | | | | |
| Aβ | -10.462 | 0.3668 | <.01 | 0.82 | -13.941 | 0.4031 | <.01 | 0.84 |
| Tau | 12.785 | 0.3384 | <.001 | | 13.829 | 0.4003 | <.01 | |
| Age | 0.0704 | 0.0251 | <.01 | | 0.0644 | 0.0296 | <.05 | |
| Gender | -0.6360 | 0.3345 | <.10 | | -0.6375 | 0.3814 | <.10 | |
| MMSE | -0.25503 | 0.0815 | <.01 | | -0.1846 | 0.0981 | <.10 | |
| Tau*Age | -0.1393 | 0.0467 | <.01 | | -0.1311 | 0.0534 | <.05 | |

CSF biomarkers (Aβ and Tau) are log-transformed and centered. MMSE: mini-mental state examination, Tau*Age: interaction between age and Tau. Interaction term was centered and standardized to allow inclusion in the model.

**Table 3.** Progression probabilities after one, three and five year

| | age | sex | MMSE | **Demographics Only** | **CSF** normal | AB abnormal | tau abnormal | both abnormal |
|---|---|---|---|---|---|---|---|---|
| **1 year** | 55 | m | 30 | 2% [0-2] | 0% [0-1] | 0% [0-2] | 12% [3-38] | 26% [7-71] |
| | | | 27 | 3% [2-5] | 0% [0-2] | 0% [1-5] | 26% [8-65] | 51% [18-92] |
| | | f | 30 | 2% [0-2] | 0% [0-1] | 0% [0-1] | 6% [2-23] | 15% [4-48] |
| | | | 27 | 3% [2-5] | 0% [0-1] | 0% [1-3] | 15% [4-44] | 32% [9-77] |
| | 70 | m | 30 | 5% [3-9] | 5% [2-15] | 13% [5-34] | 4% [1-11] | 8% [3-20] |
| | | | 27 | 11% [7-16] | 12% [5-30] | 29% [11-64] | 9% [3-21] | 19% [9-36] |
| | | f | 30 | 5% [3-9] | 3% [1-9] | 7% [2-22] | 3% [1-6] | 5% [2-12] |
| | | | 27 | 11% [7-16] | 7% [2-22] | 16% [5-46] | 5% [2-12] | 10% [4-24] |
| **3 year** | 55 | m | 30 | 4% [2-8] | 0% [0-2] | 1% [1-6] | 32% [10-78] | 60% [20-98] |
| | | | 27 | 9% [6-14] | 1% [0-5] | 2% [0-12] | 60% [23-95] | 89% [44-99] |
| | | f | 30 | 4% [2-8] | 0% [0-1] | 1% [0-3] | 19% [5-55] | 39% [11-87] |
| | | | 27 | 9% [6-14] | 0% [0-3] | 1% [0-7] | 39% [12-84] | 69% [26-99] |
| | 70 | m | 30 | 15% [10-21] | 16% [6-39] | 35% [14-72] | 13% [4-27] | 23% [11-46] |
| | | | 27 | 28% [21-37] | 33% [14-71] | 64% [30-96] | 24% [10-49] | 48% [28-71] |
| | | f | 30 | 15% [10-21] | 9% [3-25] | 21% [7-53] | 7% [3-16] | 16% [6-29] |
| | | | 27 | 28% [21-37] | 21% [7-53] | 43% [15-85] | 14% [5-32] | 29% [14-50] |

| | | | Demographics Only | CSF | | | |
|---|---|---|---|---|---|---|---|
| age | sex | MMSE | | normal | AB abnormal | tau abnormal | both abnormal |
| **5 year** | | | | | | | |
| 55 | m | 30 | | 6% | 1% | 1% | 43% | 73% |
| | | | | [3-11] | [0-3] | [0-8] | [13-89] | [27-98] |
| | | 27 | | 12% | 1% | 4% | 73% | 96% |
| | | | | [8-20] | [1-7] | [1-17] | [30-99] | [56-100] |
| | f | 30 | | 6% | 0% | 1% | 25% | 51% |
| | | | | [3-11] | [0-2] | [0-4] | [7-69] | [16-94] |
| | | 27 | | 12% | 1% | 2% | 50% | 81% |
| | | | | [8-20] | [0-4] | [0-10] | [17-93] | [35-98] |
| 70 | m | 30 | | 20% | 22% | 47% | 17% | 32% |
| | | | | [14-29] | [9-51] | [19-84] | [6-36] | [16-58] |
| | | 27 | | 38% | 44% | 78% | 32% | 60% |
| | | | | [29-49] | [19-83] | [39-98] | [24-62] | [38-83] |
| | f | 30 | | 20% | 13% | 28% | 10% | 21% |
| | | | | [14-29] | [4-34] | [10-66] | [5-22] | [8-38] |
| | | 27 | | 38% | 28% | 55% | 20% | 39% |
| | | | | [29-49] | [10-66] | [20-94] | [10-39] | [20-66] |

Biomarker values were selected as 90th percentile (normal; -) and 10th percentile (abnormal; +), for Tau 10th percentile was selected as normal (-) and 90th percentile as abnormal (+). Note that this table is an example, as the model can provide individualized risk estimates for any given value. Data are % [95% CI]. For CSF -/-: AB and TAU negative, +/-: AB positive, TAU negative, -/+: AB negative, TAU positive, +/+ AB and TAU positive.

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# CHAPTER 5

Apolipoprotein A1 in Cerebrospinal Fluid and Plasma and Progression to Alzheimer's Disease in Non-Demented Elderly

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## ABSTRACT

**Introduction**: HDL-cholesterol transporter Apolipoprotein A1 (ApoA1) holds neuroprotective properties, such as inhibition of Amyloid Beta aggregation. Low plasma ApoA1 concentrations are associated with Alzheimer's Disease (AD). Little is known about ApoA1 levels in the pre-dementia stages of AD.

Objective: To investigate associations between CSF and plasma ApoA1 levels and clinical progression towards AD in non-demented elderly.

**Methods**: From the Amsterdam Dementia Cohort we included 429 non-demented elderly with Subjective Cognitive Decline (SCD; N=206, 61±9 years, MMSE 28±2) and Mild Cognitive Impairment (MCI; N=223, 67±8 years, MMSE 27±2)), with a mean follow-up of 2.5±1.6 years. We used Cox proportional hazard models to investigate relations between CSF and plasma ApoA1 concentrations and clinical progression, defined as progression to MCI or AD for SCD, and progression to AD for MCI. Analyses were adjusted for age, gender, MMSE and plasma cholesterol levels. Analyses were stratified for diagnosis and APOE e4 carriership.

**Results**: 117 patients (27%) showed clinical progression. One standard deviation increase of CSF ApoA1 was associated with a 30% increased risk of clinical progression (Hazard Ratio (95% CI)=1.3(1.0-1.6)). The effect appeared to be attributable to the APOE e4 carriers with SCD (HR 3.3(1.0-10.9)). Lower plasma ApoA1 levels were associated with an increased risk of clinical progression in APOE e4 carriers with SCD (HR 5.0(1.3-18.9)).

**Discussion**: Higher CSF and lower plasma ApoA1 levels were associated with an increased risk of clinical progression in APOE e4 carriers with Subjective Cognitive Decline; suggesting that ApoA1 may be involved in the earliest stages of AD.

## INTRODUCTION

The e4 allele of the Apolipoprotein E gene (APOE) is a major genetic risk factor for Alzheimer's Disease (AD) [1–3], and seems to influence, besides Apolipoprotein E (ApoE) concentrations, the expression of other apolipoproteins as well [4,5]. Recent proteomic studies have identified Apolipoprotein A1 (ApoA1) as being related to Alzheimer pathology [6,7]. ApoA1 is mainly involved in reverse cholesterol transport, preventing atherosclerosis by transporting excessive cholesterol back to the liver [8]. ApoA1 is, next to ApoE, the second most abundant apolipoprotein in cerebrospinal fluid (CSF), present in HDL-like particles and maintaining cholesterol homeostasis in the brain [8–10]. ApoA1 is probably transported over the blood brain barrier by transcytosis, facilitated by the Scavenger Receptor class B type 1 (SRB1) [11], but it is also expressed by brain capillary endothelial cells [12–14]. ApoA1 has been shown to inhibit Amyloid-ß (Aß) aggregation and prevent Aß induced neurotoxicity in vitro [15–17]. ApoA1 deficiency in AD mouse models lowered plasma cholesterol and increased cerebral amyloid angiopathy (CAA) and cognitive deficits, but it did not alter parenchymal amyloid deposition [17,18]; whereas overexpression of ApoA1 in APP/PS1 mice did not prevent amyloid deposition, but preserved cognitive function and attenuated CAA [19].

In clinical studies, reduced plasma ApoA1 levels have been observed in AD patients compared with controls [20-22]. In MCI, low plasma ApoA1 was the strongest predictor for cognitive decline out of several apolipoproteins [5]. In a community based study, the combination of APOE e4 genotype and low plasma ApoA1 levels was associated with an increased risk of AD [4]. A few previous studies have reported on ApoA1 levels in CSF, comparing AD patients and controls, and results were not consistent [23-29]. Moreover, no data on CSF ApoA1 in pre-dementia stages of AD were available.
Neurodegenerative changes, eventually leading to dementia due to AD, begin to accumulate at least twenty years before clinical symptoms appear [30,31]. In the search for the underlying mechanism of the disease, the field is gradually moving forward in the disease process. Mild Cognitive Impairment (MCI) refers to the stage where patients experience memory impairment, but perform within normal limits on tests for global cognition and function independently at home [32,33]. Subjective cognitive decline (SCD) refers to individuals who experience cognitive deterioration despite normal functioning on cognitive testing. This entity has recently been suggested as a potential first symptomatic expression of AD [34,35].

We aimed to investigate the association between ApoA1 levels in CSF and plasma and clinical progression in non-demented patients with MCI and SCD. In addition, we evaluated whether these associations were modulated by APOE e4 carriership. We found that higher CSF and lower plasma ApoA1 were associated with an increased risk of clinical progression in pre-dementia patients, particularly in APOE e4 carriers with subjective cognitive decline.

## MATERIALS AND METHODS

### Subjects

From the Amsterdam Dementia Cohort we included 452 non-demented patients with a baseline diagnosis of SCD or MCI, with available CSF and plasma and at least one year follow up. All patients underwent a standardized dementia screening including neuropsychological, physical and neurologic examination as well as laboratory tests, electro-encephalography (EEG) and brain magnetic resonance imaging (MRI) [36]. Diagnoses were made in a multidisciplinary consensus meeting. All MCI patients fulfilled NIA-AA core clinical criteria for MCI [32,33]. Patients were labeled as having SCD when they presented with memory complaints, but cognitive functioning was normal and criteria for MCI, dementia or any other neurological or psychiatric disorder known to cause cognitive decline were not met [35]. At the yearly follow-up visit in the memory clinic patient history, cognitive tests and physical and neurologic examination were repeated and diagnoses were re-evaluated. All AD patients fulfilled NIA-AA core clinical criteria for dementia due to AD [37]. The main outcome measure was clinical progression. In MCI, clinical progression was defined as progression to dementia due to AD. In patients with SCD, clinical progression was defined as progression to MCI or dementia due to AD. If patients with SCD first progressed to MCI and then to AD, the moment of progression to MCI was taken as time of clinical progression. Twenty-two patients that progressed to another form of dementia than AD were excluded. Furthermore, we excluded one patient with an ApoA1 CSF concentration of six standard deviations (SD) above the mean. This resulted in 206 patients with SCD and 223 with MCI.

### Ethics, consent and permissions

The local medical ethics committee of the VU University Medical Center approved collection of data and biomaterial from patients for research purposes. All patients gave written informed consent for the use of their data in research. All research was conducted in accordance with the Helsinki Declaration of 1975.

### APOE genotyping

APOE genotyping was performed after automated genomic DNA isolation from 7-10 mL EDTA blood. It was subjected to PCR, checked for size and quantity using a QIAxcel DNA Fast Analysis kit (Qiagen, Venlo, The Netherlands) and sequenced using Sanger sequencing on an ABI130XL. Subjects with at least one e4 allele were classified as APOE e4 carriers, patients without an e4 allele were considered as non-carriers.

### CSF and plasma biomarker analyses

CSF and plasma analyses were performed at the Neurochemistry Laboratory at the department of Clinical Chemistry of the VU University Medical Center Amsterdam. CSF and plasma were collected from non-fasted subjects. CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space by a 25-gauge needle and collected in polypropylene tubes.

The first 2.5 mL CSF was collected in a separate tube for cell (erythrocyte) counting (expressed as number of erythrocytes/ 3μL CSF) to determine possible blood contamination of the CSF. Eighty-seven % of the available CSF samples had erythrocyte counts lower than 1500, corresponding to - based on average ApoA1 CSF (3 μg/mL) and plasma (1 mg/ml) levels - a contribution of plasma apoA1 to the CSF apoA1 levels of 3.8% at the maximum. This CSF sample was also used to determine AD biomarker levels (Amyloid ß1-42, Tau and phosphorylated Tau 181 (pTau)), after centrifugation at 1,800x g, using ELISA (Innotest, Fujirebio, Ghent, Belgium) [38]. The interassay CVs obtained were 11.3% (4.9) for Aß42, 9.3% (1.5%) for Tau, and 9.4% (2.5%) for pTau. Staff involved in AD-biomarker analysis was blinded for clinical diagnosis. EDTA plasma was collected in 7 mL tubes. CSF and plasma for biobanking were centrifuged, aliquotted into 0.5 mL vials and stored at -80 degrees Celsius until further analysis. A maximum of 2 h was allowed between collection and freezing [39].

ApoA1 levels in CSF (n=401) and EDTA plasma (n=411) were measured using a commercial sandwich ELISAPRO kit for human ApoA1 (Mabtech, Nacka Strand, Sweden). This assay utilizes ELISA strips pre-coated with capture monoclonal antibody (HDL110), to which samples are added. Captured ApoA1 is detected by adding another, biotinylated ApoA1 specific monoclonal antibody (HDL44). Concentration in the sample is determined by comparison to a serial dilution of purified human ApoA1, resulting in a standard range of 0.32 – 31.6 ng/ml. Pools of surplus routine plasma samples selected to have either high or low apoA1 concentrations were run as a quality control. Plasma and CSF samples were tested at 1:100.000 and 1:1000 dilutions, respectively. Intra- assay CV's for ApoA1 results were on average 2.3% for plasma and 4.0% for CSF samples. Inter-assay CV's (26 plates) were 13.0% and 9.1% for the low and high ApoA1 plasma controls, respectively.

Plasma lipid levels were measured with a Modular P system (Roche, Mannheim, Germany). For total cholesterol, HDL-cholesterol, and triglycerides, the following reagents were used: CHOD-PAP, HDL-C plus, and GPO-PAP, respectively (Roche, Mannheim, Germany). The inter-assay CVs were all less than 7%. LDL-cholesterol was calculated using the Friedewald formula [39]. Cholesterol measurements were available for 411 of 429 patients.

**Neuropsychological assessment**
We used standardized measurements to assess cognitive functioning. Of the standardized test battery we used total immediate recall and delayed recall of the Dutch version of the Rey auditory verbal learning task (RAVLT) to evaluate memory function [40]. To evaluate executive function we used Trail Making Test (TMT) B, and for global cognition we used the Mini Mental State Examination (MMSE) [41,42].

**Statistical Analysis**
Data were analyzed using SPSS for Macintosh, version 20 (IBM, Armonk, NY). Groups were compared using t-tests and chi-squared tests as appropriate. All biomarkers were log-transformed, because they did not have a normal

distribution. Subsequently, we transformed values to z-scores. We used Cox proportional hazard models to assess associations between CSF and plasma ApoA1 concentrations and clinical progression to MCI or dementia due to AD. CSF and plasma ApoA1 were used as independent variables (separate models), and clinical progression was used as outcome measure. Plasma ApoA1 values were inverted prior to analysis. Results are presented as Hazard Ratio (HR) (95% Confidence Interval). HR's represent the risk of clinical progression associated with one standard deviation (SD) increase in CSF ApoA1 concentration or one SD decrease in plasma ApoA1. We cumulatively adjusted for age, sex, MMSE (model 1), and total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides (model 2). To assess the effect of the combination of CSF and plasma ApoA1 on clinical progression we performed Cox proportional hazard models with the CSF/plasma ApoA1 ratio. The CSF/plasma ApoA1 ratio was calculated based on raw ApoA1 scores, followed by transformation to z-scores. Additionally, all analyses were repeated for SCD and MCI separately, and we re-ran all analyses stratified for APOE e4 carriership.

To assess the effects ApoA1 on clinical progression in confirmed Amyloid positive patients, we repeated Cox proportional hazards models in non-demented individuals with CSF Aß42 levels below a cut-off of 550 ng/L, based on previous studies of our center [38].

We used multivariate regression analyses with forward selection to identify the combination of biomarkers most predictive of cognitive function. Predictive variables were: Aß42, tau, pTau, plasma ApoA1 and CSF ApoA1. We adjusted analyses for age and gender age, gender. Dependent variables were MMSE, Immediate and delayed recall (RAVLT), TMT-A and B (in separate models). We reported associations between the biomarkers and cognitive markers as standardized Beta.

Correlations between CSF and plasma ApoA1 levels and between ApoA1 concentrations and plasma cholesterol levels, CSF Aß42, Tau and pTau levels and age were assessed using Pearson's correlation coefficient. P <0.05 was considered significant.

## RESULTS

Table 1 shows the baseline characteristics. On average, patients were 64±9 years of age, 180 (42%) were female and they had a mean MMSE of 27±2. 213 (50%) of the patients were APOE e4 carriers. There was a mean follow up was 2.5±1.6 years. During follow-up, 117 patients (27%; 26 SCD and 91 MCI) showed clinical progression. Patients with clinical progression were older, had lower MMSE scores and were more frequently APOE e4 carriers. Patients showing clinical progression had lower CSF Aß42 and higher CSF Tau and pTau concentrations at baseline, than those who remained stable. There were no differences in CSF or plasma ApoA1 levels between patients with clinical progression vs. patients that remained stable in the total group. In the SCD subgroup CSF ApoA1 and the CSF/plasma ApoA1 ratio was higher in patients that showed clinical progression than stable individuals, see table 2.

We used Cox proportional hazard models to investigate relationships between CSF and plasma ApoA1 levels and the risk of clinical progression. Table 2 shows the Hazard Ratio's (95% Confidence Interval). Adjusted for age, sex and MMSE, CSF ApoA1 was associated with an increased risk of clinical progression in the total group; as an increase of one SD in CSF ApoA1 was associated with a 30% increased risk of clinical progression (HR (95% CI)= 1.3 (1.0-1.6)). Additional adjustment of the models for HDL-, LDL- and total cholesterol did not change the effect. Stratification for APOE e4 genotype revealed that the effect was attributable to APOE e4 carriers (HR (95%CI)= 1.4 (1.1-18), compared to APOE e4 non-carriers HR 1.1 (0.7-1.6)). There was no relation between plasma ApoA1 and the risk of clinical progression in the total group. We then repeated the Cox proportional hazard models with the CSF/plasma ApoA1 ratio to assess the relation with clinical progression. A higher CSF/plasma ApoA1 ratio was associated with an increased risk of clinical progression in non-demented elderly (HR (95%CI)= 1.14 (1.04-1.24), adjusted for age, sex and MMSE).

Subsequently, we re-ran all models in SCD and MCI patients separately (table 2). We found that the observed effects were mostly attributable to SCD patients. In the SCD group, higher CSF ApoA1 was associated with an increased risk of clinical progression (HR (95% CI)= 1.5 (0.9-2.4)), although not significant. After stratification for APOE genotype the effect of CSF ApoA1 on progression was most prominent in the APOE e4 carriers (HR (95% CI)= 2.7 (1.1-6.5), adjusted for age, sex and MMSE; and HR (95% CI)= 3.3 (1.0-10.9), additionally adjusted for cholesterol concentrations). Lower plasma ApoA1 was associated with an increased risk of clinical progression in APOE e4 carriers with SCD (HR (95% CI)= 5.0 (CI 1.3-18.9), after adjustment for age, sex, MMSE and cholesterol concentrations; plasma ApoA1 scores were inverted prior to Cox proportional hazards analyses). For the CSF/plasma ApoA1 ratios, effects were most prominent in APOE e4 positive individuals with SCD (HR (95% CI)= 1.63 (1.11-2.40)). In MCI, we found no significant associations between either CSF or plasma ApoA1 and clinical progression.

To assess the effects of CSF and plasma ApoA1 on clinical progression in Amyloid positive patients (CSF Aβ1-42 value below 550 ng/L), we repeated Cox proportional hazards models in non-demented Amyloid positive individuals (N=143, 29% of total group), but results were not significant anymore.

Correlations between CSF and plasma ApoA1 concentrations, the CSF/plasma ApoA1 ratio, plasma cholesterol values and CSF Alzheimer biomarkers are summarized in Table 3. We found no correlation between CSF and plasma ApoA1 (r=0.03, p=0.594) for the total group, and also when cases with clinical progression towards the AD trajectory at follow up (N=117; r= -.189, p=0.057) and cases that remained clinically stable (N=312; r=0.097, p=0.104) were considered separately (Figure 1). Plasma ApoA1 was strongly related to HDL cholesterol, and moderately with total cholesterol and triglycerides. CSF and plasma ApoA1 did not correlate with CSF Aβ1-42, but there was a weak correlation between plasma ApoA1 and CSF Tau, largely attributable to the MCI group. There was a modest positive correlation between CSF ApoA1 and age.
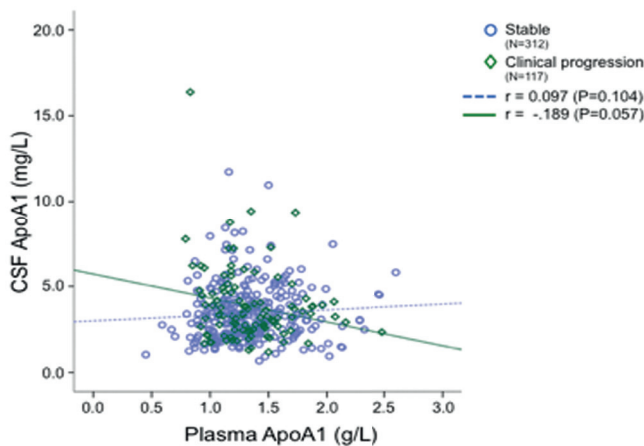


Figure 1. Scatterplot of the relation between CSF and plasma ApoA1 levels in non-demented elderly (N=429), of whom 117 clinically progressed towards the AD trajectory at follow up (green) and 312 cases remained clinically stable (blue).

We used multivariate regression analyses with forward selection to identify the best combination of biomarkers associated with individual cognitive markers (MMSE, Immediate and delayed recall (RAVLT), and TMT-B). Results are displayed in table 4. The optimal model for global cognition (MMSE) included CSF Tau and CSF ApoA1. The optimal model for both immediate and delayed recall included CSF Tau, CSF Aβ1-42 and plasma ApoA1, but after adjustment for age and gender plasma ApoA1 was excluded from the best fitted model. For executive functioning the optimal model included CSF Tau and CSF Aβ1-42.

## DISCUSSION

The main finding of this study is that higher baseline CSF ApoA1 was associated with an increased risk of clinical progression in non-demented APOE e4 carriers with SCD. In these patients, lower levels of plasma ApoA1 were also associated with increased risk of clinical progression. To further assess the coherence between these two biomarkers, we looked into the CSF/plasma ApoA1 ratio. This ratio was higher in patients that progressed towards dementia than in patients that remained stable, but only in SCD these differences were significant. The increased CSF/plasma ratio in progressors reflects the relatively higher CSF ApoA1 and lower plasma ApoA1 levels associated with an increased risk of progression in individual patients. This, and the percentage-wise larger change in CSF (Table 1), suggests that higher CSF ApoA1 contributes relatively more to progression than lower plasma ApoA1 in our population.

Former studies have shown reduced plasma ApoA1 levels in patients with AD compared to controls [20–22]. Low plasma ApoA1 was also identified as the apolipoprotein with the strongest predictive value for cognitive decline in patients with MCI [5]. In the Honolulu aging study, decreased plasma ApoA1 was related to clinical progression in a community based sample, particularly in APOE e4 carriers [4]. Our findings are in line with these former studies, as we found that low plasma ApoA1 concentrations were associated with an increased risk of clinical progression. In our study the effect was specific for subjects in the earliest stages of AD (i.e. subjective cognitive decline), and particularly the APOE e4 carriers.

Few previous studies on CSF ApoA1 in relation to AD are available. Available studies mainly reported reduced ApoA1 levels in patients with AD compared to controls, but results were not consistent. Some studies showed decreased levels of CSF ApoA1 [26–29], while others showed no effect [24] or increased CSF ApoA1 [25] in patients with AD versus healthy controls. In our sample of pre-dementia patients, we observed an association between increased concentrations of CSF ApoA1 and an increased risk of clinical progression towards AD. Differences in results for CSF ApoA1 between our study and previous studies may be due to methodological issues, as well as to differences in disease stage of subjects included in the different studies. Concerning methodological issues, results in former studies were not adjusted for age, sex or other potential confounders, possibly because of their small sample sizes [25–28]. In addition, in some studies CSF was obtained by lumbar puncture [24-27], while in others ventricular CSF was obtained post-mortem [28,29], which makes the comparison of protein concentrations difficult [43]. Concerning differences in disease stage, previous studies on CSF ApoA1 compared only AD patients with healthy controls, while we studied pre-dementia patients [26–29]. It is conceivable that levels increase in the earliest phase of AD, and decrease later in the disease process. Support for this notion comes from our findings that specifically in subjects with SCD, elevated CSF ApoA1 levels were associated with an increased risk of clinical progression, while in MCI we did not observe this effect.

Limitations of the study include the relative short mean follow up of 2.5 years in view of a disease process that may take at least 20 years. The number of subjects with cognitive decline at follow-up was relatively small in the group of patients with SCD, and as a result Cox models did not converge in some of the smaller strata (APOE e4). Future studies should include longitudinal measurements of ApoA1 to investigate changes in CSF and plasma ApoA1 values over time in relation to cognitive decline. A previous study indicated that blood contamination of CSF samples, measured as CSF Hemoglobin concentration, could influence reliability of CSF measurements [44]. In our study we used available erythrocyte concentration to assess possible blood contamination of the CSF samples. The majority (>87%) of available CSF samples had erythrocyte levels lower than 1500/ 3 μl CSF, corresponding to a maximal contribution of plasma apoA1 to the CSF apoA1 level of 3.8% (0.135 μg/ml). Therefore, and because of possible overestimation of erythrocyte numbers as these were determined in the first 2.5 ml collected that was not used for storage in our biobank, we considered the influence of possible blood contamination on our CSF results a minor problem. Strengths of the current study are the longitudinal design and highly standardized clinical follow-up. To our knowledge this is the first study to assess such a large sample of paired CSF and plasma ApoA1 data, in combination with CSF AD biomarkers, in the pre-dementia stages of AD.

A lack of association between specific biomarker concentrations in CSF and plasma has been reported for AD biomarkers, for example Aβ1-42 [45]. CSF and plasma ApoA1 concentrations probably do not correlate, because ApoA1 is produced in the liver and small intestine, and is also expressed by brain capillary endothelial cells, but not in the CNS itself [13,14]. The blood brain barrier separates these compartments, and therefore concentrations in plasma and the brain might be independent from each other. On the other hand, it has also been suggested that ApoA1 is possibly transported over the blood brain barrier by transcytosis, facilitated by the Scavenger Receptor class B type 1, suggesting an influence of plasma ApoA1 concentrations on ApoA1 in cerebro [11]. Further research is needed to assess the impact of this transport over the blood brain barrier on cerebral and CSF concentrations of ApoA1.

ApoA1 has been shown to inhibit Aβ aggregation and also to exert protective effects against Aβ mediated neurotoxicity in vitro [15–17], as well as in animal models for AD [17,19]. In ApoA1 deficient AD mice, ApoA1 levels in brain and CSF were reduced, and, possibly as a compensatory mechanism, plasma ApoE levels, but not CSF ApoE levels, were increased [18]. ApoA1 deficient AD mouse models exhibited memory deficits to a certain degree which paralleled cerebral vascular Aβ accumulation [17], whereas overexpression of ApoA1 in AD mice attenuated cognitive deficits and reduced the degree of CAA and neuroinflammation [19]. This protective effect of ApoA1 against neuroinflammation has also been previously described in Parkinson's disease [46]. The beneficial effects of increased ApoA1 plasma levels on cerebral amyloid deposition [19] may be due to ApoA1 binding to Aβ, and preventing its toxic effects on vascular smooth muscle

cells, since ApoA1, lipidated as well as non-lipidated, was found to protect brain vascular smooth muscle cells from Aß in vitro [17]. In AD mouse models, ApoA1 levels were not related to Aß accumulation in the brain parenchyma [17–19]. Thus, although ApoA1 has been suggested to influence Aß metabolism [15], its main effect seems to be on vascular Aß accumulation. Cerebral amyloid deposition has been observed at neuropathological examination in 23-45% of the non-demented elderly [47]. It can be suggested that vascular amyloid deposition may be an early phenomenon in the pathophysiology of AD, which, as judged from the AD mouse model studies [17–19], can be associated with ApoA1 expression levels. The early increase in CSF ApoA1 observed in our study in non-demented patients, may be a protective measure in the earliest stages of AD, in which ApoA1 can exert neuroprotective effects, especially when Aß-mediated [15–17,19].

Because of the intimate involvement of Aß in the pathogenesis of AD and CSF Aß1-42 levels being reduced in very early stages of AD, we next looked if ApoA1 levels were associated with AD biomarkers in CSF, but found no strong associations between CSF and plasma ApoA1, and the AD biomarkers CSF Aß42 and Tau (Table 3). The significance of the relation between CSF and plasma apoA1 and AD biomarkers observed in the total group, was lost when Cox proportional hazard models for CSF and plasma ApoA1 were repeated after stratification for CSF Abeta1-42 values (above or below the cut off of 550 ng/L, indicative of AD), probably due to the small group sizes.

Further research on the possible influence of apolipoproteins on Alzheimer pathology is currently ongoing in our cohort using mediation analyses. We also investigated how CSF and plasma ApoA1, in combination with CSF Tau and Aß42, were aligned with cognitive measurements. The optimal model of biomarkers explaining global cognition included CSF Tau, Aß42 and CSF ApoA1. The optimal model explaining the memory domain included CSF Tau, CSF Aß42 and plasma ApoA1, but after adjustment for age and gender plasma ApoA1 was excluded. Lower plasma ApoA1 was related to worse cognition, which is in line with previous research indicating that lower plasma ApoA1 concentrations were associated with worse cognitive performance and severity of AD [22,48].
Although associations between CSF ApoA1 concentrations and CSF Aß42 values have not been studied before, research on APOA1 polymorphisms showed that presence of the APOA1 -75bp A allele may be associated with lower CSF Aß42, and also with increased risk of AD [48,49]. It would be of interest to further assess the contribution of APOA1 polymorphisms on ApoA1 concentrations in relation to Alzheimer biomarkers, within the light of other apolipoproteins and APOE e4 carriership.
We found stronger effects for both CSF and plasma ApoA1 in APOE e4 carriers than non-carriers, especially in SCD. APOE e4 carriership has been suggested to influence the pathophysiological sequences leading to dementia due to AD [3,50], and is associated with reduced CSF Aß42 concentrations in patients with AD [51,52]. After stratification for e4 carriership, effects of plasma ApoA1 were

different in e4-carriers vs. non-carriers, which is similar to the results of two previous studies in which the effect of plasma ApoA1 on progression towards AD differed between APOE e4 carriers and non-carriers with normal cognition [4,5]. We now indicate that also for CSF ApoA1 concentrations, effects seem to differ between APOE e4 carriers and non-carriers. How APOE e4 carriership exerts its effects on ApoA1 remains to be elucidated.

In conclusion, both higher CSF ApoA1 and lower plasma ApoA1 are associated with an increased risk of clinical progression in the pre-dementia AD, especially in subjects with SCD and APOE e4 carriership, suggesting a role for ApoA1 in the earliest stages of AD.

**Table 1.** Baseline characteristics of the study population

| | Total group | | | Subgroups | | | | | |
| | | | | Subjective Cognitive Decline | | | Mild Cognitive Impairment | | |
| | All | Stable | Clinical progression | All | Stable | Clinical progression | All | Stable | Clinical progression |
|---|---|---|---|---|---|---|---|---|---|
| N | 429 | 312 | 117 | 206 | 180 | 26 | 223 | 132 | 91 |
| Age, y | 64.1±9.0 | 62.4±8.8 | 68.7±7.9** | 61.0±8.8 | 60.3±8.8 | 66.0±7.6** | 67.1±8.2** | 65.4±8.0 | 69.5±7.9** |
| Sex, female | 180 (42%) | 122 (39%) | 58 (50%) | 86 (42%) | 77 (43%) | 9 (35%) | 94 (42%) | 45 (34%) | 49 (54%)** |
| MMSE | 27.4±2.2 | 27.7±2.0 | 26.5±2.5 | 28.3±1.6 | 28.3±1.6 | 27.9±1.7 | 26.6±2.4** | 26.9±2.2 | 26.1±2.6* |
| Follow-up duration, y | 2.5±1.6 | 2.6±1.6 | 2.3±1.5* | 2.8±1.7 | 2.8±1.7 | 2.5±2.1 ns | 2.3 ± 1.3** | 2.4±1.4 | 2.2±1.3 |
| APOE e4 carrier, n(%) | 213(50%) | 137(44%) | 79(68%)** | 86(42%) | 70 (39%) | 16(61%)* | 130(58%)** | 67(51%) | 63(69%)* |
| CSF AB$_{42}$, ng/L | 730±280 | 810±267 | 515±186** | 834±245 | 865±226 | 618±265** | 633±276** | 735±299 | 486±146** |
| CSF Tau, ng/L | 406±311 | 312±211 | 654±391** | 296±205 | 277±196 | 428±224** | 506±356** | 360±222 | 718±405** |
| CSF pTau, ng/L | 63±33 | 54±26 | 87±37** | 50±23 | 49±22 | 64±28** | 74±37** | 61±30 | 93±37** |
| Total cholesterol mmol/L | 5.5±1.1 | 5.5±1.0 | 5.6±1.2 | 5.5±1.0 | 5.5±1.0 | 5.5±1.1 | 5.5± 1.1 | 5.4±1.1 | 5.6±1.1 |
| HDL cholesterol mmol/L | 1.5±0.5 | 1.5±0.5 | 1.5±0.5 | 1.6±0.5 | 1.6±0.5 | 1.5±0.6 | 1.5±0.5 | 1.5±0.5 | 1.5±0.5 |
| LDL cholesterol mmol/L | 3.3±1.0 | 3.3±0.9 | 3.4±1.1 | 3.3±0.9 | 3.3±0.9 | 3.2±1.0 | 3.3±1.0 | 3.2±0.9 | 3.4±1.1 |
| Triglycerides mmol/L | 1.5±0.9 | 1.5±0.9 | 1.5±0.9 | 1.5±0.9 | 1.5±0.9 | 1.8±1.2 | 1.5±0.9 | 1.5±1.0 | 1.5±0.7 |
| CSF ApoA1 mg/L | 3.5±1.8 | 3.4±1.7 | 3.8±2.1 | 3.4±1.6 | 3.3±1.5 | 4.2±2.1* | 3.6±1.9 | 3.6±1.8 | 3.7±2.1 |
| Plasma ApoA1 g/L | 1.4±0.3 | 1.4±0.3 | 1.4±0.3 | 1.4±0.4 | 1.4±0.4 | 1.3±0.4 | 1.3±0.3 | 1.3±0.3 | 1.4±0.3* |
| CSF/plasma ApoA1 ratio | 2.8±1.7 | 2.7±1.4 | 3.1±2.4 | 2.7±1.4 | 2.6±1.3 | 3.7±2.0* | 2.9±2.0 | 2.9±1.6 | 3.0±2.4 |

Data are presented as mean ± standard deviation or n (%), *: p<0.05; **: p<0.01. T-test and chi-square test were used to assess differences between diagnostic groups (SCD vs. MCI) and patients remaining stable vs. patients progressing towards Alzheimer's Disease within diagnostic groups. CSF=cerebrospinal fluid, ApoA1=Apolipoprotein A1, MMSE=mini mental state examination, AB=Amyloid Beta, pTau=phosphorylated Tau. Cholesterol values were measured in plasma.

**Table 2.** Associations between Apolipoprotein A1 in Cerebrospinal Fluid and Plasma and clinical progression in non-demented elderly

| | | All | p | SCD | p | MCI | p |
|---|---|---|---|---|---|---|---|
| | | | | **Subgroups** | | | |
| **CSF ApoA1** | Model 1 | 1.30 (1.04-1.63) | .023 | 1.49 (0.91-2.42) | .110 | 1.15 (0.88-1.50) | .298 |
| | APOE e4 neg | 1.04 (0.69-1.56) | .870 | 1.16 (0.56-2.41) | .685 | 0.86 (0.49-1.54) | .619 |
| | APOE e4 pos | 1.38 (1.05-1.81) | .021 | 2.66 (1.10-6.47) | .031 | 1.20 (0.9-1.60) | .213 |
| | Model 2 | 1.30 (1.03-1.63) | .030 | 1.42 (0.85-2.37) | .182 | 1.18 (0.90-1.54) | .236 |
| | APOE e4 neg | 1.06 (0.70-1.62) | .776 | Did not converge | | 0.97 (0.53-1.78) | .923 |
| | APOE e4 pos | 1.37 (1.04-1.81) | .024 | 3.34 (1.30-10.86) | .045 | 1.20 (0.90-1.61) | .218 |
| **Plasma ApoA1** | Model 1 | 0.96 (0.78-1.18) | .702 | 0.99 (0.62-1.58) | .980 | 0.89 (0.70-1.14) | .379 |
| | APOE e4 neg | 1.01 (0.73-1.38) | .974 | 0.71 (0.28-1.76) | .456 | 1.00 (0.69-1.46) | .981 |
| | APOE e4 pos | 0.96 (0.72-1.28) | .787 | 1.32 (0.71-2.46) | .384 | 0.85 (0.61-1.20) | .360 |
| | Model 2 | 1.05 (0.73-1.51) | .804 | 1.99 (0.97-4.10) | .059 | 0.87 (0.57-1.33) | .523 |
| | APOE e4 neg | 1.15 (0.62-2.14) | .666 | Did not converge | | 1.13 (0.53-2.41) | .751 |
| | APOE e4 pos | 1.02 (0.63-1.67) | .927 | 5.04 (1.34-18.91) | .016 | 0.76 (0.45-1.29) | .309 |
| **CSF/ plasma ApoA1 ratio** | Model 1 | 1.27 (1.08-1.50 | .004 | 1.39 (0.99-1.98) | .066 | 1.18 (0.95-1.47) | .126 |
| | APOE e4 neg | 1.11 (0.74-1.64) | .620 | 1.13 (0.61-2.06) | .702 | 1.05 (0.53-2.07) | .894 |
| | APOE e4 pos | 1.28 (1.08-1.52) | .005 | 2.01 (1.15-3.49) | .014 | 1.17 (0.94-1.45) | .160 |
| | Model 2 | 1.30 (1.10-1.55) | .003 | 1.44 (0.99-2.10) | .056 | 1.23 (1.00-1.52) | .054 |
| | APOE e4 neg | 1.15 (0.77-1.73) | .489 | Did not converge | | 1.33 (0.61-2.86) | .475 |
| | APOE e4 pos | 1.31 (1.09-1.58) | .005 | 3.04 (1.15-8.04) | .025 | 1.19 (0.95-1.50) | .131 |

Data are presented as Hazard Ratio (HR) (95% confidence interval). ApoA1 concentrations were log-transformed and transformed to z-scores prior to analysis. For CSF HR's represent the increased risk of clinical progression for each SD increased level of CSF ApoA1. Note that plasma levels are inverted; as a result HR's represent increased risk of clinical progression for each SD lower level of plasma ApoA1. CSF/plasma ApoA1 ratio was calculated based on raw ApoA1 scores followed by transformation to z-scores.

**Table 3.** Correlations between Apolipoprotein A1 and cholesterol, CSF Alzheimer biomarkers, MMSE and age

| ApoA1 in | All | | | Subgroups | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | Subjective Cognitive Decline | | | Mild Cognitive Impairment | | |
| | CSF | Plasma | CSF/plasma ratio | CSF | Plasma | CSF/plasma ratio | CSF | Plasma | CSF/plasma ratio |
| Plasma ApoA1 | .03 | | | .03 | | | .02 | | |
| Total cholesterol | -.05 | .36** | -.26** | -.03 | .41** | -.26** | -.07 | .32** | -.26** |
| HDL cholesterol | -.01 | .82** | -.33** | -.03 | .82** | -.38** | .01 | .81** | -.28** |
| LDL cholesterol | -.07 | .13* | -.20** | -.02 | .17* | -.20** | -.11 | .08 | -.19** |
| Triglycerides | .08 | -.32** | .17 | .13 | -.39** | .25** | .05 | -.25** | .10 |
| CSF Aβ$_{42}$ | .04 | -.05 | .01 | .13 | -.04 | .00 | .03 | -.10 | .02 |
| CSF tau | .07 | .10* | .00 | .03 | .11 | -.02 | .12 | .16* | .02 |
| CSF ptau | .05 | .07 | -.01 | .02 | .04 | -.01 | .08 | .15* | -.01 |
| Age | .13* | .06 | .08 | .07 | .11 | .07 | .16* | .06 | .10 |

Results are displayed as Pearson's correlation coefficient (r), *: p<0.05; **: p<0.01. ApoA1=Apolipoprotein A1, Aβ42=Amyloid Beta1-42, pTau=phosphorylated Tau.

**Table 4.** Significant associations between CSF biomarkers and cognitive measurements in non-demented elderly, identified with multivariate regression analysis with forward selection.

| | Global cognition (MMSE) | | | Memory | | | | | | Executive function (TMT) | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | Immediate recall | | | Delayed recall | | | | | |
| | | Standardized Beta | Adjusted $R^2$ | | sBeta | Adj. $R^2$ | | sBeta | Adj. $R^2$ | | sBeta | Adj. $R^2$ |
| Biomarker | CSF Tau | -.15* | .052 | CSF Tau | -.25** | .148 | CSF Tau | -.31** | .199 | CSF Tau | .20* | .076 |
| | CSF ApoA1 | .14* | | CSF A$\beta_{42}$ | .20** | | CSF A$\beta_{42}$ | .23** | | CSF A$\beta_{42}$ | -.14* | |
| | CSF A$\beta_{42}$ | .11* | | Plasma ApoA1 | .17** | | Plasma ApoA1 | .14* | | | | |
| Biomarker, adjusted for age and sex | CSF Tau | -.15* | .054 | CSF Tau | -.23** | .166 | CSF Tau | -.28** | .208 | CSF Tau | .17* | .090 |
| | CSF ApoA1 | .13* | | CSF A$\beta_{42}$ - | .17** | | CSF A$\beta_{42}$ | .21** | | | | |
| | CSF A$\beta_{42}$ | .11* | | | | | | | | | | |

Results are displayed as standardized Beta. * P<0.05, **P<0.001. All biomarker values and neuropsychological tests were log transformed and transformed to Z-scores prior to analysis. Higher scores on MMSE, immediate and delayed recall, and lower scores on Trail making test indicated better cognition. ApoA1 = Apolipoprotein A1, Aß42= Amyloid Beta1-42.

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# CHAPTER 6

ApoE and clusterin CSF levels influence
associations between APOE genotype and
changes in CSF tau, but not CSF amyloid-beta 1-42,
levels in non-demented elderly
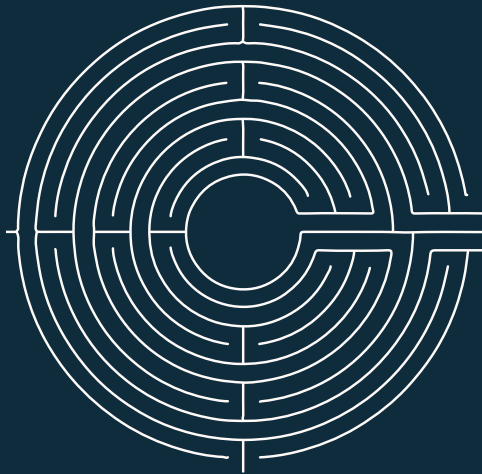
**Slot R.E.R.**, Kester M.I., Van Harten A.C., Jongbloed W., Bouwman F.H.,
Teunissen C.E., Scheltens P., Van der Flier W.M., Veerhuis R.

**ABSTRACT**

APOE e4 genotype is associated with increased cerebral amyloid-beta (Abeta) deposition in non-demented elderly, and suggested to influence apopolipoprotein E (ApoE), as well as Apo-J (clusterin) and Apo-A1, expression. We aimed to assess whether APOE affects early AD pathophysiology via these apolipoproteins. CSF ApoE, clusterin, ApoA1, and CSF Abeta 42 and tau levels were assessed in 403 individuals with Subjective Cognitive Decline and Mild Cognitive Impairment, using ELISA. Whether CSF apolipoprotein levels mediated APOE e4 allele frequency effects on CSF Abeta 42 and tau in non-demented elderly, was investigated using mediation analysis, with age and gender adjusted linear regression analyses. CSF ApoE mediated 48% of the association between APOE e4 and CSF tau, whereas clusterin and ApoA1 did not. Additionally, CSF clusterin partially mediated the relation between CSF ApoE and tau (12%). CSF apolipoproteins did not mediate the inverse relation between APOE e4 and CSF Abeta42, despite a strong association between the latter two biomarkers. In summary, our findings suggest that ApoE and clusterin are involved in Abeta-independent pathways as part of the cascade leading to Alzheimer pathology.

## INTRODUCTION

Apolipoprotein E (APOE) e4 genotype is a major genetic risk factor for Alzheimer's Disease (AD), associated with an earlier age of onset of dementia [1]. The APOE gene encodes for the protein apolipoprotein-E (ApoE), which regulates lipid homeostasis in the brain and also supports injury repair [1]. In the CNS, ApoE is primarily produced by astrocytes and microglia [2]. The three ApoE isoforms (e2, e3 and e4) have different isoform specific binding affinities for specific lipids and amyloid beta (Abeta) [3]. APOE e4 carriers have more cerebral amyloid deposition than subjects with an e3 or e2 isoform, and e4 carriership is associated with lower CSF amyloid beta1-42 (Abeta42) concentrations [4–6]. APOE e4 carriership may increase the risk of AD via effects on Abeta clearance, either through effects on transport to the blood stream or on glial cell uptake, or modulation of Abeta–induced glial cell activation [7]. On the other hand, ApoE might influence AD pathophysiology also via Abeta-independent mechanisms, including its anti-inflammatory properties, its effects on alterations in neurovasculature or the ApoE e4 isoform related deficits in cholesterol homeostasis, affecting synaptic integrity and plasticity [8,9].

With the lack of therapeutic options once a diagnosis of dementia due to AD is made, pre-dementia and preclinical stages of AD have become a focus of research [10]. In non-demented elderly APOE e4 carriership is associated with an increased prevalence of amyloid positivity [11]. Patients with AD have altered levels of ApoE in CSF and plasma compared to individuals with normal cognition and MCI, although no consensus has been reached whether higher or lower ApoE levels are associated with AD [12–15]. In non-demented elderly, altered CSF and plasma ApoE levels are associated with an increased risk of AD, an effect most prominently observed in APOE e4 carriers [16–18]. Also for other apolipoproteins, namely apolipoprotein A1 (ApoA1) and apolipoprotein J (also referred to as clusterin (Clu)), alterations in CSF and plasma levels have been associated with an increased risk of progression to dementia in non-demented elderly, and in some studies these effects were more prominent in APOE e4 carriers, too [17,19–22]. Besides ApoE, Clu is the main brain cholesterol transporter. In HDL particles Clu co-localizes with ApoA1, the third most abundant apolipoprotein in the central nervous system, and mainly involved in the reverse cholesterol transport in peripheral tissues [2]. Both Clu and ApoA1 have been suggested to affect Abeta-deposition and clearance [4,23–25], and are considered to have neuroprotective properties, whereas Clu has been reported to be compensatory induced in response to low brain levels of ApoE in APOE e4 carriers [23,26–30].

To investigate whether APOE affects the early pathophysiology of AD via apolipoproteins, we assessed whether the association between APOE e4 allele frequency and CSF Abeta42 and tau levels could be explained by mediation of these associations by CSF ApoE, Clu and ApoA1 levels, in non-demented elderly with Subjective Cognitive Decline and Mild Cognitive Impairment.

## METHODS

### Subjects
From the Amsterdam Dementia Cohort, we included 403 non-demented patients with available CSF and a baseline diagnosis of Subjective Cognitive Decline (SCD, N=191) or Mild Cognitive impairment (MCI, N=212) [31,32]. All patients underwent a standardized dementia screening including neuropsychological, physical and neurologic examination as well as laboratory tests, electro-encephalography (EEG) and brain magnetic resonance imaging (MRI) in a memory clinic setting [33]. Diagnoses were made in a multidisciplinary consensus meeting. Patients were labeled as having SCD when they presented with memory complaints, but cognitive functioning was normal, and criteria for MCI, dementia or any other neurological or psychiatric disorder known to cause cognitive decline were not met [31]. MCI was diagnosed according to Petersen's criteria, and all MCI patients fulfilled NIA-AA core clinical criteria for MCI [32,34].

### Ethical procedures
The local medical ethics committee of the VU University Medical Center Amsterdam approved the collection of data and biomaterial from patients for research purposes. All patients gave written informed consent for the use of their data and biomaterial for research purposes. All research was conducted in accordance with the Helsinki Declaration of 1975.

### Biomarker measurements
*APOE genotyping*
APOE genotyping was performed after automated genomic DNA isolation from 7-10 mL EDTA blood. It was subjected to PCR, checked for size and quantity using a QIAxcel DNA Fast Analysis kit (Qiagen, Venlo, The Netherlands) and sequenced using Sanger sequencing on an ABI130XL.

*CSF Abeta42 and tau analyses*
CSF analyses were performed at the Neurochemistry Laboratory at the department of Clinical Chemistry of the VU University Medical Center Amsterdam. CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space by a 25-gauge needle and collected in polypropylene tubes (Sarstedt, Numbrecht, Germany). 2,5 mL CSF was used for routine analyses, and Amyloid-beta1-42 (Abeta42), tau and tau phosphorilyzed threonine 181 (ptau) were measured using ELISA (Innotest, Fujirebio, Ghent, Belgium) [35]. CSF for biobanking was centrifuged, aliquotted into 0.5 mL polypropylene vials (Sarstedt) and stored at -80 degrees Celsius until further analysis [36].

*CSF apolipoprotein analyses*
ApoA1 concentrations in CSF were measured using a commercial sandwich ELISAPRO kit for human ApoA1 (Mabtech AB, Nacka Strand, Sweden; Cat. No.: 3710-1HP-10), according to the manufacturer's instructions. This assay utilizes ELISA strips pre-coated with capture monoclonal antibody (HDL110), to

which samples were added. Captured ApoA1 was detected by adding another, biotinylated ApoA1 specific monoclonal antibody (HDL44). Serial dilutions of purified human ApoA1 were used to prepare a standard curve (range 0.1 – 100 ng/ml) to calculate concentrations in the samples. CSF samples were tested at 1:1000 dilutions. Intra-assay coefficients of variance (CV's) for CSF ApoA1 results were on average 4.0%. Inter-assay CV's (26 plates) were 13.0% and 9.1% for the low and high ApoA1 plasma controls, respectively.

ApoE concentrations in CSF were measured using a commercial sandwich ELISA (Mabtech AB, Nacka Strand, Sweden; Cat. No.: 3712-1H-20). Monoclonal antibody (E276) was used to capture the ApoE present in the samples. Captured ApoE was subsequently detected by another biotinylated ApoE specific monoclonal antibody (E887). Serial three-fold dilutions of recombinant ApoE3 in Assay buffer (Mabtech AB) were used to prepare a standard curve ranging from 0.03 – 31.6 ng/ml. CSF samples were diluted 1:2000 in Assay buffer. Intra-assay CV's for CSF ApoE results were on average 3.0%. Inter-assay CV's (24 plates): 10.3 % and 10.4 % for plasma high and low respectively.

Clusterin concentrations in CSF were determined in a sandwich ELISA using a combination of clusterin-specific mouse monoclonal antibodies (kindly provided by dr. Braesch-Andersen; Mabtech AB, Nacka Strand, Sweden). Antibody J29 was used to capture clusterin in samples, and another, biotinylated mouse monoclonal antibody, J84, for detection. Clusterin to prepare a standard curve (range 0.11 – 285 ng/ml), was isolated by affinity chromatography using clusterin–specific monoclonal antibody G7 coupled to Sepharose 4B beads [22]. The intra-assay coefficient of variance was below 5%. Inter-assay CV was 9.8%. Detection of ApoA1, ApoE and clusterin in the ELISAs was visualized upon subsequent incubations with streptavidin-HRP and 3,5,3',5'-tetramethylbenzidine (TMB; Sigma, Germany).

**Statistical Analyses**
In this cross-sectional study data were analyzed using SPSS for Macintosh, version 20 (IBM, Armonk, NY). Demographic features were compared based on APOE e4 carriership, and diagnosis (SCD vs. MCI), using T-tests or Chi-squared tests as appropriate. Prior to analyses all biomarkers were log-transformed, because they did not have a normal distribution. Subsequently, we transformed all biomarker values to z-scores.

Associations between APOE e4 allele frequency, CSF Abeta42 and tau levels, and CSF ApoE, Clu and ApoA1 concentrations were investigated using linear regression analyses, adjusted for age and gender. Subsequently, we used mediation analyses to assess whether CSF apolipoproteins influenced (mediated) the relation between APOE e4 allele frequency and CSF Abeta42 and tau. Mediation analysis is a method to evaluate the possible influence of mediator M on the relation between independent variable (X) and outcome variable (Y) [37–39]. We used linear regression analysis, adjusted for age and gender, to evaluate associations between independent variable X (APOE e4 allele frequency) and outcome variable Y (CSF Abeta42 or tau), this direct association was referred to as relation c. Then we evaluated associations

between X and possible mediator M (i.e. CSF ApoE, clusterin or ApoA1) (association X-M was referred to as association a), and the association between M and Y adjusted for X (referred to as association b). To evaluate possible mediation by mediator M on the relation between X and Y, associations a, b and c need to be significant, otherwise one cannot evaluate mediation. Finally, we evaluated the amount of change in regression coefficient (direct association X-Y (c)) adjusted for mediator M, resulting in the indirect association X-Y (referred to as association c'). The mediation effect was then calculated (c-c') as well as the percentage of mediation ((c-c')/c*100) which represents the amount by which mediator M explains the relation between X and Y. See also figure 1 for a visual representation of the mediation concept. With regards to terminology we used the word mediate to indicate a statistical mediation by mediator M on the relation between X and Y. This does not automatically imply a causal relation, but does indicate a statistical influence of M on the association between X and Y.

Besides APOE e4 allele frequency, we took CSF ApoE as a starting point and evaluated mediation of CSF Clu and ApoA on the association between CSF ApoE and CSF tau.

All analyses were adjusted for age and gender, $P < 0.05$ was considered significant.
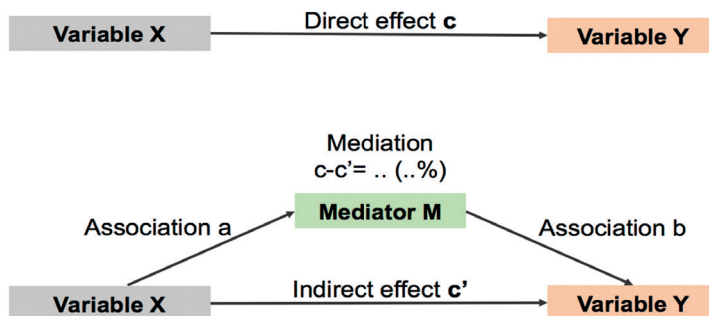


Figure 1. Concept of mediation. Visual interpretation of the concept of mediation. X= independent variable, Y= outcome variable, M= (possible) mediating variable. To evaluate possible mediation by mediator M on the relation between X and Y, variable X and Y and variable X and mediator M need to be significantly associated, otherwise one cannot speak of mediation. a= association between variable X and M; b=association between mediator M and Y, adjusted for variable X; c=association between X and Y; c'= association between X and Y, adjusted for mediator M. Mediation= c-c', percentage of mediation = (c-c')/c*100.

## RESULTS

### Demographic features and CSF AD biomarker and apolipoprotein levels

Table 1 shows the demographic features of the study participants. Distribution of APOE e4 allele frequency (0/1/2 alleles) was 203/142/58. On average patients were 64±9 years of age, with a mean MMSE of 27±2, and 166 (41%) were female. APOE e4 carriers had lower CSF Abeta42, higher CSF tau and higher CSF ApoE levels (3.1±1.4 vs. 3.9±3.1, p=.000). CSF Clu and ApoA1 concentrations did not differ between APOE e4 carriers and non-carriers. CSF ApoE, Clu and ApoA1 were all associated with age (respectively Beta 0.18, p=.000; 0.26, p=.000 and 0.14, p=0.006). There were no gender differences in CSF ApoE and Clu levels, whereas ApoA1 levels were lower in female participants (3.2±1.5 vs 4.0±4.1, p=.000). Therefore, all analyses were adjusted for age and gender.

### Associations between APOE e4, CSF Abeta42, tau and CSF apolipoproteins

Table 2 shows associations between APOE e4 allele frequency, CSF Abeta42, tau and CSF apolipoproteins. We assessed these relations between APOE e4 allele frequency and CSF analytes using linear regression analysis, adjusted for age and gender. Increased APOE e4 allele frequency was associated with lower CSF Abeta42 (Beta -.62, p=.000), higher CSF tau (.31, p=.000) and higher CSF ApoE levels (.41, p=.000), but not CSF Clu or ApoA1. Higher CSF tau was associated with higher CSF ApoE (.44, p=.000) and higher CSF Clu levels (.49, =.000), but not ApoA1. Meanwhile, CSF Abeta42 was not associated with any of the CSF apolipoproteins we measured. See figure 2 for scatterplots of associations between CSF Abeta42 and tau, and CSF apolipoproteins.
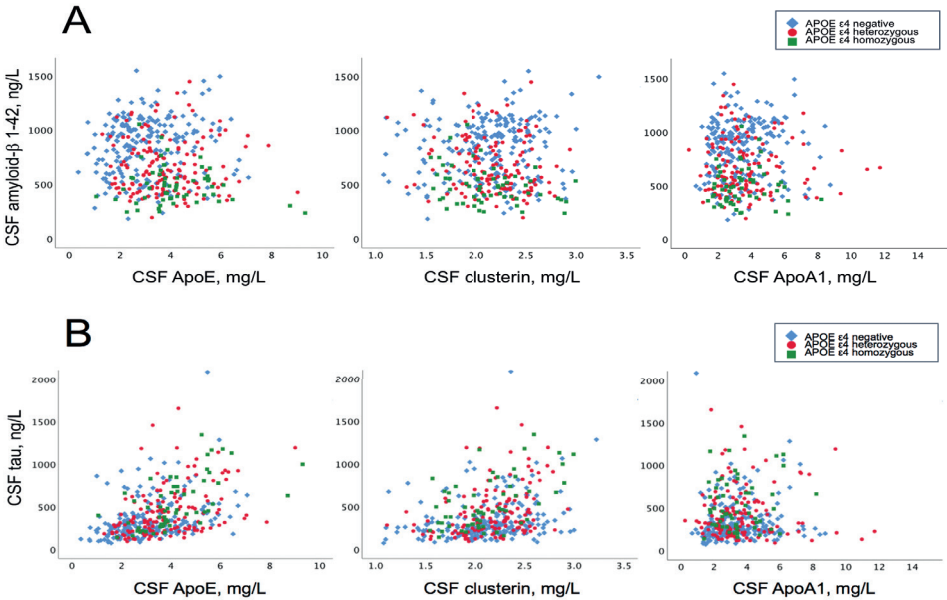


Figure 2. Associations between CSF Abeta42, tau and apolipoproteins. 2A scatterplots of associations between CSF Abeta42 and CSF ApoE, clusterin and ApoA1, marked by APOE e4 status (APOE e4 negative, e4 heterozygous or homozygous). 2B scatterplots of associations between CSF tau and CSF ApoE, clusterin and ApoA1, marked by APOE e4 status.

Higher CSF Clu was associated with both higher CSF ApoE (Beta 0.42, p=.000) and higher CSF ApoA1 (0.37, p=.000), but there was no relation between CSF ApoE and ApoA1. After stratification for diagnosis (SCD or MCI) results remained essentially the same, only the association between APOE e4 allele frequency and CSF tau, and Abeta42 and CSF tau was not significant anymore in individuals with SCD (respectively 0.11, p=.161 and -.07, p=.255, see table 2).

**Mediation analyses of CSF apolipoproteins on the relation between APOE e4 allele frequency and CSF Abeta42 and tau.**

We investigated mediation of CSF apolipoproteins using linear regression analyses, adjusted for age and gender. The direct association between APOE e4 allele frequency and CSF Abeta42 was -0.62 (c; p=.000). CSF ApoE, Clu or ApoA1 did not mediate this relation (see figure 3). For more detailed results of linear regression analyses used to evaluate mediation of CSF apolipoproteins see supplementary table A. The direct association between APOE e4 allele frequency and CSF tau was 0.31 (c; p=.000). This relation was partially mediated by CSF ApoE (c'; Beta 0.16, p=.000; c-c'=0.15, Beta ratio 48%, see figure 4). CSF Clu or ApoA1 did not mediate the association between APOE e4 allele frequency and CSF tau. Taking CSF ApoE as a starting point, the association between CSF ApoE and CSF tau was 0.41 (c; sBeta, p<0.001). This relation was partially mediated by CSF Clu (c'; sBeta 0.36, p<0.001; c-c'=0.05, Beta ratio 12%), but not by ApoA1, see figure 5.

After stratification for diagnosis (SCD vs. MCI) all results remained essentially the same. Only the association used to evaluate mediation of CSF ApoE on the relation APOE e4 allele frequency and CSF tau was not significant anymore, but results were still in the same direction. For more detailed results after stratification for diagnosis see supplementary table A.
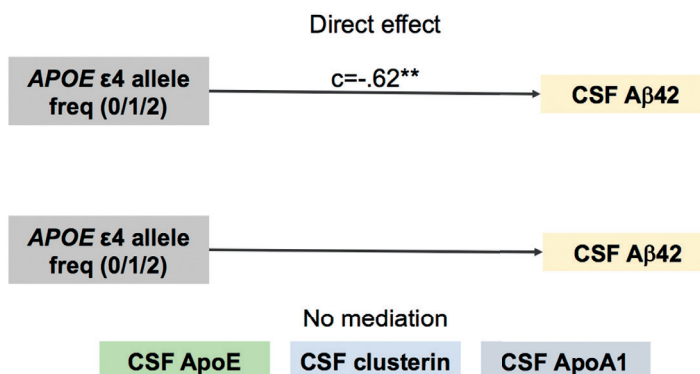


Figure 3. No mediation by apolipoproteins on relation APOE e4 allele frequency and CSF A¤42. No mediation by apolipoproteins of the association between APOE e4 allele frequency and CSF A¤42. Results displayed as standardized Beta, adjusted for age and gender, ** p<0.001. A¤42 = amyloid-beta1-42, ApoE = apolipoprotein E, ApoA1 = apolipoprotein A1, freq = frequency, c = association adjusted for age, gender.

ApoE and clusterin CSF levels mediate APOE genotype effects on Alzheimer's disease related changes in CSF tau, but not CSF Abeta42, in non-demented elderly
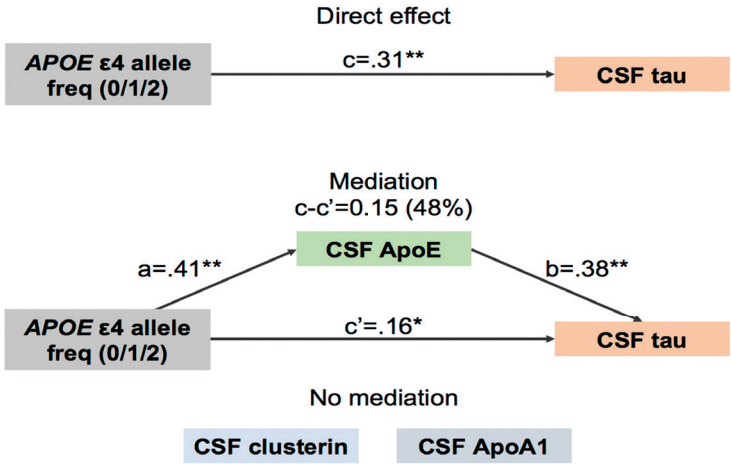


Figure 4. Mediation by apolipoproteins on relation APOE e4 allele frequency and CSF tau. Mediation (48%) by CSF ApoE of the association between APOE e4 allele frequency and CSF tau. CSF clusterin and ApoA1 do not mediate the association. Results displayed as standardized Beta, adjusted for age and gender, * p<0.05, ** p<0.001. ApoE = apolipoprotein E, ApoA1 = apolipoprotein A1, freq = frequency, c = association adjusted for age, gender, c' = association adjusted for age, gender and mediator.
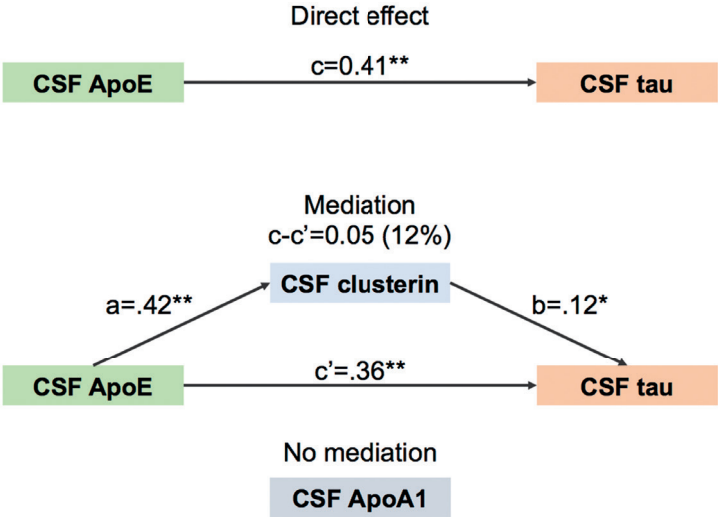


Figure 5. Mediation by apolipoproteins on relation CSF ApoE and CSF tau. Mediation (12%) by CSF clusterin of the association between CSF ApoE and CSF tau. Results displayed as standardized Beta, adjusted for age and gender, * p<0.05, ** p<0.001. ApoE = apolipoprotein E, ApoA1 = apolipoprotein A1, c = association adjusted for age, gender, c' = association adjusted for age, gender and mediator.

## DISCUSSION

APOE e4 carriership is a major genetic risk factor for Alzheimer's disease, but the exact pathophysiological mechanisms eventually leading to AD remain to be elucidated. To assess whether APOE e4 genotype exerts its effect on AD pathology directly via ApoE levels, or indirectly via effects on levels of other apolipoproteins (Clu or ApoA1), that share some of the lipid- and Abeta-carrier properties of ApoE, we examined the mediating effects of these apolipoproteins in CSF of non-demented elderly. We found that CSF ApoE levels partially explained the relation between APOE e4 allele frequency and CSF tau, and that CSF levels of apolipoprotein Clu mediated the association between CSF ApoE and tau. Contrary to our expectation, CSF ApoE protein levels did not mediate the relation between APOE e4 allele frequency and CSF Abeta42, despite a strong association between these latter two biomarkers.

Previous studies observed APOE e4 allele dose-dependent effects on the risk of developing AD and amyloid plaque load in the brain, with an inverse effect of APOE e4 allele frequency on CSF Abeta42 levels [5,6]. Effects of APOE e4 may be due to effects on clearance and degradation of Abeta42, as well as effects on A¤ production [40,41]. In line with previous research, we found a strong negative association between APOE e4 allele frequency and CSF Abeta42 levels. However, the strong relation between APOE e4 and CSF Abeta42 levels could not be explained by changes in CSF ApoE levels, despite the association of APOE e4 allele frequency with both CSF Abeta42 and CSF ApoE levels in our cohort. The lack of association between CSF ApoE and CSF Abeta42 levels we observed, is in contrast to some [42–44], but not other [15,24,45] studies.
We did find a dose dependent effect of APOE e4 allele frequency on CSF tau levels, for which CSF ApoE levels were a substantial mediator. Clinical studies have reported associations between CSF tau and CSF ApoE levels in patients with AD [15,46], but direct effects of APOE e4 carriership on tau in AD are less frequently studied than associations with Abeta42. In vitro the ApoE e4 isoform was found to induce tau hyperphosphorylation, while ApoE e3 increased the functional activity of protein phosphatase 2, which dephosphorylates phosphorylated tau in neurofibrillary tangles, suggesting opposite effects of e3 and e4 isoforms on AD related neuronal damage [47]. Furthermore, ApoE e4 has been shown to interact with cytoskeletal proteins to form tangle-like structures containing phosphorylated tau, and truncated ApoE e4 fragments might increase cytoskeletal disruption and mitochondrial dysfunction and neurotoxicity [48,49].

The lack of association between CSF ApoE and CSF Abeta42 levels in our study, together with the mediation of CSF ApoE in the relation between APOE e4 and tau, may suggest that the ApoE e4 isoform influences neuronal damage, reflected by increased CSF tau levels, indirectly via other mechanisms independent of Abeta. It has been proposed that tau-related pathology, independent of Abeta42, might initiate and contribute to the pathogenesis of AD [50,51].

Possible Abeta-independent pathways affecting the pathophysiology of AD, include the influence of APOE on inflammatory processes, cerebrovascular changes, and lipid homeostasis and thereby synaptic plasticity, amongst others [8]. We theoretically evaluated these possible underlying mechanisms provoking AD pathophysiology independent of Abeta. First, innate immunity maintains homeostasis in cerebro through clearance of aged and/or obsolete proteins and cells, but can also initiate neuronal damage when not properly controlled [52]. The ApoE e4 isoform has reduced anti-inflammatory properties compared to the other isoforms, which facilitates a pro-inflammatory environment with elevated levels of pro-inflammatory cytokines TNF-alpha and IL-6 [53,54], and increased microglial activation, which in turn may facilitate neuronal damage [52,54].

*Second*, cerebrovascular effects of ApoE include effects on integrity of the blood-brain barrier (BBB), which is more impaired in AD patients carrying one or two APOE e4 alleles, and may contribute to the disease via impaired Abeta clearance [55]. Independent of A□, BBB dysfunction may affect the pathophysiology of AD via impaired brain microcirculation, inducing neuronal dysfunction and injury [56]. How ApoE is involved in impaired vascular integrity and BBB dysfunction still remains largely unknown. A likely scenario suggested that expression of ApoE e4 can lead to activation of a proinflammatory cyclophilin A-nuclear factor-κB (Nf-κB)-matrix-metalloproteinase-9 (MMP-9) pathway in pericytes, that results in BBB breakdown [57], and ultimately to neuronal dysfunction. Combined, these studies suggest that independent of Abeta, ApoE might influence vascular integrity early in AD pathogenesis.

A *third* Abeta-independent scenario beholds the influence of APOE on lipid homeostasis. Cholesterol is a vital component of cell membranes, and maintaining lipid homeostasis is of great importance in the prevention of neurodegenerative diseases [2]. ApoE mediated lipid redistribution is indispensable to the maintenance of synapse integrity and plasticity, both known to be affected in AD [58]. ApoE is mainly produced by astrocytes and microglia. Neuronal ApoE expression is upregulated after neuronal damage, possibly to induce neuronal repair [59]. The ApoE e4 isoform is thought to confer the risk of AD via less effective lipidation and neuronal cholesterol delivery (Bu, 2009), which may lead to impaired neuronal plasticity and reduced neurogenesis, both involved in the pathogenesis of AD, independent of Abeta [8]. While the ApoE e4 isoform is less efficient in lipid transport, other apolipoproteins, such as Clu, may in turn be able to facilitate lipid transport and compensate for the ApoE e4 isoform associated loss in function. A graphical overview of associations and hypothetical underlying changes is provided in Figure 6.

When we took the relationship between CSF ApoE and CSF tau as a starting point, we found that this relation was partially mediated (12%) by CSF Clu levels. A possible explanation is that Clu expression is upregulated in response to neuronal damage, in our study reflected by a higher Clu levels associated with higher tau. In a mouse model overexpressing tau, Clu expression was found to be upregulated in the brain, while intracellular Clu interacted with

tau in neurons [60]. However, in previous clinical studies we have observed a correlation between CSF tau and clusterin levels not only in non-demented patients frequenting our Alzheimer center [22], but also in non-demented Parkinson patients and neurologically healthy controls [61], which suggests that Clu may be involved in physiologic processes as well. In the present study we observed a relation between CSF Clu and CSF tau, but no significant association between CSF Clu and CSF Abeta42, whereas we did see a relation between CSF Clu and CSF Abeta42 in MCI cases, but not in AD or SCD, before (Jongbloed et al., 2015). Clu probably plays a physiological role in the clearance of Abeta42 towards the CSF through complex formation. The difference in associations between CSF Clu and CSF Abeta42 between our previous and present studies may be due to the extent of Clu-Abeta complex formation that may differ between health and disease, and may interfere with CSF Abeta-measurements [62], thus influencing associations between CSF Clu and CSF Abeta42 levels determined in ELISAs. The composition of the non-demented elderly group in the current study comprised MCI and also SCD cases, and thus more cases without AD pathology. Moreover, the group size in the current study was 8-fold larger, and adjusted for age and gender, which may all to some extent have contributed to the observed differences in association between current and previous studies. Clu promotes neuroprotective or regenerative processes, including neurite outgrowth and network complexity in reaction to neuronal damage, a capacity thought to be impaired in the ApoE e4 isoform [63,64]. Therefore, and because in brain homogenates of AD patients with APOE e4 carriership ApoE levels were lower and Clu higher, it has been suggested that Clu substitutes ApoE when levels or functional activity of ApoE are reduced [30]. Such a substitution of ApoE by Clu may explain the mediating effect of Clu on the CSF ApoE and tau association.

Previous research indicated that ApoA1 was of influence in pre-dementia stages of AD [17,19,20], but we found no mediating effect of CSF ApoA1 on relations between APOE e4 carriership and CSF Abeta42 or tau. In our study CSF ApoA1 was associated with CSF Clu, possibly because both co-localize in HDL particles, but ApoA1 did not relate to either CSF ApoE, Abeta or tau levels. ApoA1 and Clu both have neuroprotective properties and may be upregulated in response to Abeta related AD pathology [23,27,28]. However, whereas CSF Clu levels probably increase due to local production in cerebro, those of ApoA1, which is mainly produced in the liver, probably increase due to enhanced transport over the blood brain barrier [65]. CSF or plasma ApoA1 have not been previously reported to be correlated with CSF or plasma Abeta42, but plasma ApoA1 levels were associated with plasma Abeta-40 in CAA patients, and apoA1 was suggested to be a physiologic transporter of soluble Abeta at the peripheral level [66]. This may differ from the situation in the brain parenchyma as Clu facilitated Abeta1-40 efflux over the BBB in an in vitro model using mouse cerebral capillary endothelial cells, whereas ApoA1 did not [25]. In summary, further research is needed to investigate the differential roles of these two apolipoproteins, both with neuroprotective properties but with distinct roles in cholesterol and presumably also Abeta transport.

Our findings suggest a differential role for ApoE and Clu in individuals with SCD and MCI with AD pathology, which are both stages in which patients are not demented. Strengths of the study include the large sample of non-demented elderly with CSF biomarkers measured following stringent procedures and extensive standardized clinical investigation in a memory clinic setting. It is of note that not all individuals with MCI, and even less with SCD, eventually progress to dementia due to AD. Memory complaints and cognitive deficits may have various other underlying causes, such as vascular cognitive impairment and depression amongst others [67,68]. Therefore, evaluation of the observed mediation effects in relation to clinical subtypes and follow up of clinical progression of participants may be of much interest. Unfortunately, the limited sample size of subsets did not allow such an evaluation. The lack of individuals with dementia due to AD in our project can be considered a limitation of this study, as we could not comprise the apolipoproteins within the whole AD continuum. Either way, evaluating non-demented patients may provide more information of the role of apolipoproteins in preclinical and prodromal stages of AD. Another limitation of the study includes the lack of experimental evaluation of the proposed associations, generated by a statistical approach using mediation analysis. Mediation analysis is a useful tool to evaluate the influence of a certain 'mediator' on a proposed relationship. However, it does not imply a direction of the relationship, nor does it imply a causal relation. Evaluation of variables by mediation analysis is purely statistical and theoretical. Therefore, further experimental evaluation of the type and extent of interaction between analytes, and also APOE genotype, is essential to further understand their impact on AD pathophysiology.

In conclusion, in non-demented elderly, CSF ApoE and Clu influenced the relation between APOE e4 allele frequency and CSF tau, but not CSF Abeta42. These findings suggest that in pre-dementia stages of AD, APOE e4 genotype may contribute to the pathophysiology of AD via Abeta-independent pathways as part of the cascade leading to Alzheimer pathology.
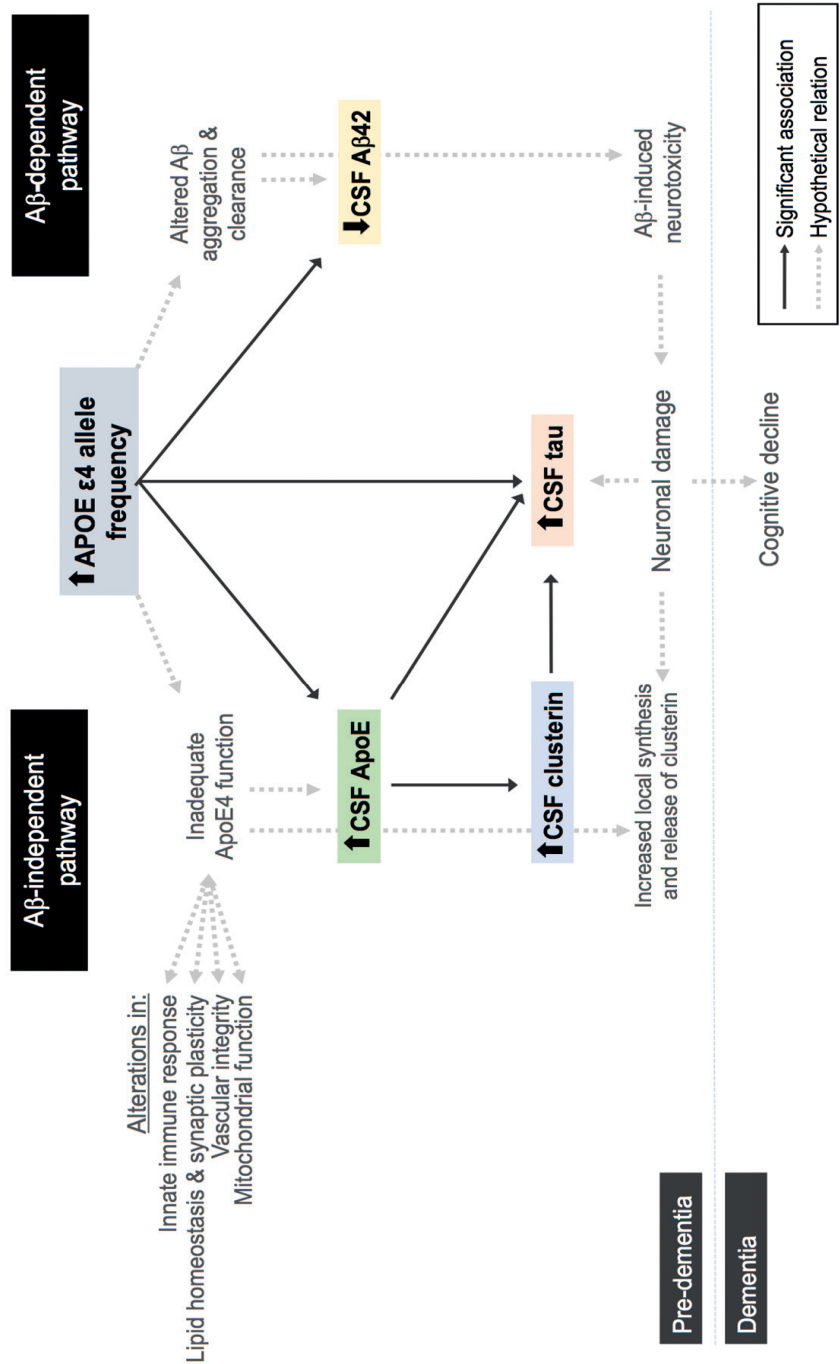
Figure 6. Overview of associations between APOE, CSF Abeta42, tau and apolipoproteins, and hypothetical underlying pathological alterations. Solid lines: significant associations, dashed lines: hypothetical relations.

**Table 1.** Demographic features of the study population of non-demented elderly (n=403)

| | All | APOE e4 negative | APOE e4 positive | P | SCD | MCI | P |
|---|---|---|---|---|---|---|---|
| N | 403 | 203 | 200 | | 191 | 212 | |
| Age, years | 64.0±9.1 | 63.3±9.5 | 64.8±8.6 | .101 | 60.5±8.8 | 67.2±8.2 | .000 |
| Gender, female | 166 (41%) | 73(36%) | 93(47%) | .034 | 75 (39%) | 91 (43%) | .479 |
| MMSE | 27.3±2.2 | 27.5±1.1 | 27.2±2.3 | .087 | 28.2±1.6 | 26.5±2.4 | .000 |
| APOE e4 positive | 200 (50%) | | | | 74 (39%) | 126 (63%) | .000 |
| APOE e4 allele frequency (0/1/2) | 203/142/58 | | | | 117/58/16 | 86/84/42 | .000 |
| CSF Aβ42, ng/L | 728±281 | 843±268 | 611±243 | .000 | 841±248 | 626±270 | .000 |
| CSF Tau, ng/L | 407±318 | 340±245 | 475±365 | .000 | 289±203 | 513±362 | .000 |
| CSF ApoE, mg/L | 3.5±1.5 | 3.1±1.4 | 3.9±3.1 | .000 | 3.3±1.4 | 3.7±1.5 | .000 |
| CSF clusterin, mg/L | 9.1±3.4 | 9.2±3.6 | 9.1±3.1 | .901 | 2.1±0.4 | 2.2±0.4 | .181 |
| CSF ApoA1, mg/L | 3.7±3.3 | 3.7±4.3 | 3.6±2.0 | .585 | 3.7±4.4 | 3.6±1.9 | .448 |

Demographic features of 403 non-demented participants with Subjective Cognitive Decline (n=191) or Mild Cognitive Impairment (n=212). Data are presented as mean ± standard deviation or n (%). T-tests and Chi-squared tests were used to assess differences between APOE e4 carriers vs. non-carriers, and between diagnoses (SCD vs. MCI). Aβ42 = amyloid beta1-42, ApoA1 = apolipoprotein A1, ApoE = apolipoprotein E, CSF = cerebrospinal fluid, MCI = mild cognitive impairment, MMSE = Mini Mental State Examination, SCD = subjective cognitive decline.

**Table 2.** Linear regression analyses between CSF analytes and APOE e4 allele frequency in non-demented elderly (n=403)

| Variable | Group | APOE e4 allele frequency (1/2/3/) | CSF Aβ$_{1-42}$' | CSF tau | CSF ApoE | CSF ApoJ (Clu) | Age |
|---|---|---|---|---|---|---|---|
| **CSF Aβ$_{1-42}$'** | All | -.62, p=.000 | | | | | -.32, p=.000 |
| | SCD | -.42, p=.000 | | | | | |
| | MCI | -.65, p=.000 | | | | | |
| **CSF tau** | All | .31, p=.000 | -.30, p=.000 | | | | .39, p=.000 |
| | SCD | .11, p=.161 | -.07, p=.255 | | | | |
| | MCI | .32, p=.000 | -.32, p=.000 | | | | |
| **CSF ApoE** | All | .41, p=.000 | -.05, p=.363 | .49, p=.000 | | | .18, p=.000 |
| | SCD | .37, p=.001 | -.02, p=.805 | .65, p=.000 | | | |
| | MCI | .41, p=.000 | -.03, p=.955 | .44, p=.000 | | | |
| **CSF ApoJ (clusterin)** | All | -.03, p=.670 | .04, p=.434 | .32, p=.000 | .42, p=.000 | | .26, p=.000 |
| | SCD | -.15, p=.152 | -.06, p=.511 | .44, p=.000 | .38, p=.000 | | |
| | MCI | .06, p=.494 | .10, p=.168 | .33, p=.000 | .48, p=.000 | | |
| **CSF ApoA1** | All | .06, p=.406 | .04, p=.428 | .07, p=.219 | .08, p=.124 | .37, p=.000 | .14, p=.006 |
| | SCD | .13, p=.285 | .09, p=.323 | -.00, p=.974 | .05, p=.492 | .40, p=.000 | |
| | MCI | .02, p=.831 | .02, p=.804 | .12, p=.073 | .10, p=.104 | .35, p=.000 | |

Overview of associations between CSF analytes and APOE e4 allele frequency in non-demented elderly (n=403), subsequently stratified for diagnosis (SCD and MCI). All linear regression analyses were adjusted for age and gender, results are displayed as Beta with P-value. We also show unadjusted associations between age and CSF analytes. Aβ1-42 = amyloid beta 1-42, Apo = apolipoprotein, Clu = clusterin, SCD = subjective cognitive decline, MCI = mild cognitive impairment.

ApoE and clusterin CSF levels mediate APOE genotype effects on Alzheimer's disease related changes in CSF tau, but not CSF Abeta42, in non-demented elderly

**Supplementary table A.** Results of linear regression analyses used for mediation analyses in non-demented elderly (n=403)

| Variable X | Variable Y | Group | Significant association X-Y (c) | Mediator M | Association X-M (a) | Association M-Y adjusted for X (b) | Association X-Y adjusted for M (c') | Mediation yes/no | Percentage of mediation ((c-c')/c) |
|---|---|---|---|---|---|---|---|---|---|
| APOE ε4 allele frequency | CSF Abeta42 | All | -.62, p=.000 | CSF ApoE | .41, p=.000 | -.04, p=.363 | -.66, p=.000 | No significant association M-Y, therefore no mediation possible | |
| | | | -.62, p=.000 | CSF Clu | -.03, p=.670 | .03, p=.510 | -.62, p=.000 | No significant association M-Y, therefore no mediation possible | |
| | | | -.62, p=.000 | CSF ApoA1 | .06, p=.406 | .06, p=.182 | -.62, p=.000 | No significant association M-Y, therefore no mediation possible | |
| | | SCD | -.42, p=.000 | CSF ApoE | See table 2, no significant association between Mediator M and variable Y (CSF Abeta42) | | -.44, p=.000 | No | |
| | | | -.42, p=.000 | CSF Clu | | " | -.43, p=.000 | No | |
| | | | -.42, p=.000 | CSF ApoA1 | | " | -.43, p=.000 | No | |
| | | MCI | -.65, p=.000 | CSF ApoE | | " | -.71, p=.000 | No | |
| | | | -.65, p=.000 | CSF Clu | | " | -.66, p=.000 | No | |
| | | | -.65, p=.000 | CSF ApoA1 | | " | -.65, p=.000 | No | |
| | CSF tau | All | .31, p=.000 | CSF ApoE | .41, p=.000 | .38, p=.000 | .16, p=.007 | Yes | c-c'=-.15 (48%) |
| | | | .31, p=.000 | CSF Clu | See table 2, no significant association between X (APOE ε4 allele freq) and Mediator M | | .32, p=.000 | No | |
| | | | .31, p=.000 | CSF ApoA1 | " | | .31, p=.000 | No | |
| | | SCD | .11, p=.161 | CSF ApoE | .37, p=.001 | .35, p=.000 | -.01, p=.854 | No | No, because association c is not significant. After adjustment for mediator association c' is clearly altered, suggesting complete mediation by mediator CSF ApoE (c-c'=.12 (complete mediation 100%)). However, no significance statistically. |

## ApoE and clusterin CSF levels mediate APOE genotype effects on Alzheimer's disease related changes in CSF tau, but not CSF Abeta42, in non-demented elderly

| Y | Group | a | Mediator M | X–M association | b | Mediation | c–c' (%) |
|---|---|---|---|---|---|---|---|
| CSF ApoE | | .11, p=.161 | CSF Clu | See table 2, no significant association between X (APOE ε4 allele freq) and Mediator M | .16, p=.044 | No | |
| | | .11, p=.161 | CSF ApoA1 | " | .12, p=.160 | No | |
| | MCI | .32, p=.000 | CSF ApoE | .41, p=.000  .39, p=.000 | .16, p=.060 | Yes | c–c'=-.15 (48%) |
| | | .32, p=.000 | CSF Clu | See table 2, no significant association between X (APOE ε4 allele freq) and Mediator M | .30, p=.000 | No | |
| | | .32, p=.000 | CSF ApoA1 | " | .32, p=.000 | No | |
| CSF tau | All | 041, p=.000 | CSF Clu | .42, p=.000  .12, p=.011 | .36, p=.000 | Yes | c–c'=0.05 (12%) |
| | | | CSF ApoA1 | See table 2, no significant association between X (CSF ApoE) and Mediator M | .41, p=.000 | No | |
| | SCD | .34, p=.000 | CSF Clu | .38, p=.000  .14, p=.012 | .29, p=.000 | Yes | c–c'=.05 (15%) |
| | | | CSF ApoA1 | See table 2, no significant association between X (CSF ApoE) and Mediator M | .35, p=.000 | No | |
| | MCI | .43, p=.000 | CSF Clu | .48, p=.000  .13, p=.050 | .36, p=.000 | Yes | c–c'=.07 (16%) |
| | | | CSF ApoA1 | See table 2, no significant association between X (CSF ApoE) and Mediator M | .42, p=.000 | No | |

Overview of linear regression analyses used to evaluate statistical mediation between analytes in 403 non-demented individuals with SCD (n=191) or MCI (n=212). All linear regression analyses are displayed as Beta with p-value, all analyses were adjusted for age and gender. All analytes were log-transformed and converted to z-scores prior to analyses. X= independent variable, Y= outcome variable, M= (possible) mediator. To evaluate possible mediation by mediator M on the relation between X and Y, variable X and Y and variable X and mediator M need to be significantly associated, otherwise one cannot speak of mediation. a= association between variable X and M, adjusted for age and gender; b=association between M and Y, adjusted for age and gender, as well as variable X; c=association between X and Y, adjusted for age and gender; c'= association between X and Y, adjusted for age, gender and variable M. SCD = subjective cognitive decline, MCI = mild cognitive impairment.

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# CHAPTER 7

Plasma Amyloid as Prescreener for the
Earliest Alzheimer Pathological Changes
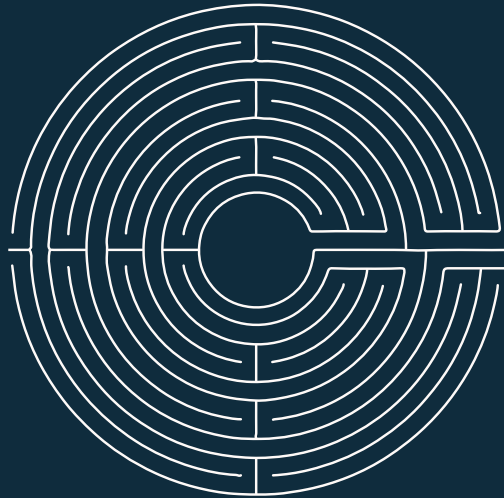
Verberk I.M.W., **Slot R.E.R.**, Verfaillie S.C.J., Heijst H., Prins N.D.,
Van Berckel B.N.M., Scheltens P., Teunissen C.E., Van der Flier W.M.

## ABSTRACT

**Introduction**: We investigated the association of plasma amyloid beta (Abeta)40, Abeta42 and total tau (t-tau) with the presence of Alzheimer's pathological changes in cognitively normal individuals with subjective cognitive decline (SCD).

**Methods**: We included 248 subjects with SCD (61±9yrs, 42%F, 28±2 MMSE) from the SCIENCe project and Amsterdam Dementia Cohort. Subjects were dichotomized as amyloid abnormal by CSF and PET. Baseline plasma Abeta40, Abeta42 and t-tau were measured using SIMOA technology. Associations between plasma levels and amyloid status were assessed using logistic regression analyses and ROC analyses. Association of plasma levels with risk of clinical progression to mild cognitive impairment (MCI) or dementia were assessed using COX proportional hazard models.

**Results**: Fifty-seven (23%) subjects were CSF-amyloid abnormal. Plasma Abeta42/Abeta40 ratio and plasma Abeta42 alone, but not t-tau, identified abnormal CSF-amyloid status (plasma ratio: AUC=77% (95%CI: 69%-84%), plasma Abeta42: AUC=66% (95%CI: 58%-74%)). Combining plasma ratio with age and APOE resulted in AUC=83% (95%CI: 77%–89%). Youden's cut-off of the plasma ratio gave a sensitivity of 76% and specificity of 75%, and applying this as pre-screener would reduce the number of lumbar punctures by 51%. Using PET as outcome, a comparable reduction in number of PET scans would be achieved when applying the plasma ratio as pre-screener. In addition, low plasma ratio was associated with clinical progression to MCI or dementia (HR=2.0 (95%CI: 1.4–2.3)).

**Discussion**: Plasma Abeta42/Abeta40 ratio has potential as pre-screener to identify Alzheimer's pathological changes in cognitively normal individuals with SCD.

**INTRODUCTION**

Alzheimer's Disease (AD) pathophysiology is hallmarked by extracellular amyloid beta (Abeta) aggregation and intracellular tau deposition, which start 10 to 20 years prior to onset of clinical symptoms.[1-3] Amyloid pathology without cognitive impairment has been defined as the earliest Alzheimer's pathological changes.[3-5] Individuals with these earliest Alzheimer's changes, i.e. abnormal amyloid status, are at increased risk of future cognitive decline[6-8] and clinical progression to dementia.[7, 9-11] For this reason, they are an important target group in the context of clinical trials that evaluate anti-amyloid therapies.

Low concentrations of Abeta in cerebrospinal fluid (CSF) as well as Abeta visualized on positron emission tomography (PET) scans have been extensively studied and have proven their accuracy to identify amyloid pathology in the brain.[3, 9, 12] The available diagnostic tools are however invasive (CSF) or expensive (PET), hampering widespread application for diagnosis, e.g. in a primary care setting, and large scale identification of individuals with an abnormal amyloid status in the context of recruitment for trials.[13] There is an urgent need for low-invasive and affordable techniques to pre-screen for cerebral amyloid pathology, subsequently forwarding less individuals towards further invasive and/or expensive testing. A blood marker would qualify as an easy pre-screening tool. Using first generation techniques like enzyme-linked immuno sorbent assays (ELISA), studies on blood Abeta led to insufficient accuracy to allow implementation in pre-screening.[12] With the recent emergence of novel high sensitive technologies the field is now quickly evolving, proving it is possible to use plasma markers to measure brain amyloid pathology.[14-17]

Although highly promising, recent studies used non-automated, labor-intensive techniques, precluding widespread implementation in large numbers of individuals.[15, 16] Others chose an automated technique but evaluated the spectrum from full-blown AD dementia to healthy controls,[14] which although essential for validation of the analytical techniques does not translate to the urgent need of easy pre-screening, which lies in the group of individuals in the very earliest stages of AD. Aiming to close this gap, we used the fully automated, highly sensitive Single Molecule Array (SIMOA) technology[18] to measure plasma concentrations of Abeta40, Abeta42 and total tau (t-tau) in a large cohort of cognitively unimpaired subjects with subjective cognitive decline (SCD). We aimed to investigate the potential value of plasma Abeta40, Abeta42 and t-tau as a pre-screening tool for abnormal cerebral amyloid status in cognitively normal individuals. To further evaluate clinical relevance of our plasma markers, we investigated their association with clinical progression to mild cognitive impairment (MCI) or dementia.

## METHODS

### Subjects
We included 248 subjects labeled as Subjective Cognitive Decline (SCD) from the ongoing Amsterdam Dementia Cohort and SCIENCe project.[19-21] All subjects visited the memory clinic of the VU University Medical Center between November 2000 and August 2016 for extensive dementia screening that consisted of neurological, physical and neuropsychological evaluation, biomarker analyses in CSF obtained by lumbar puncture, electroencephalography and brain magnetic resonance imaging.[19, 20] Subjects were labeled as SCD upon multidisciplinary consensus, when no abnormalities on clinical or cognitive testing were observed and criteria for MCI, dementia or other medical conditions potentially causing cognitive decline were not met (i.e. no psychiatric diagnosis).[4, 22] Inclusion criteria for this study were met when baseline CSF biomarker data and EDTA plasma sample collected within 0.5 years from baseline visit were available, and at least one follow-up visit was performed. Written consent to use medical data and biomaterials for research purposes was in place, in accordance with the ethical consent by the VU University and with the Helsinki Declaration act of 1975.

### Clinical progression
Subjects were followed on an annual basis (mean follow-up $3\pm2$ years) where neurological, physical and neuropsychological examination was repeated. Based on these results, the diagnosis was re-evaluated by clinical consensus. Clinical progression was defined as a change in diagnosis to MCI (Petersen criteria until 2012,[23] and NIA-AA criteria for MCI from 2012 onwards[24]), to Alzheimer's Dementia (NINCDS-ADRDA until 2011,[25] and NIA-AA criteria for AD from 2011 onwards[26]) or other types of dementia.[27-30] Time to clinical progression was calculated as the date difference between baseline blood sampling and the date at which clinical progression was first diagnosed. When SCD subjects progressed to MCI first and later to dementia, the date on which MCI was first diagnosed was used to estimate time to clinical progression.

### Amyloid status
CSF concentrations of Abeta42, t-tau and tau phosphorylated at threonine 181 (pTau181) were measured using Innotest ELISAs (Fuijirebio, Ghent, Belgium) by trained technicians who were blinded for clinical diagnosis.[31] CSF Abeta42 levels were adjusted for the drift in CSF biomarker analyses that occurred over the years and subsequently dichotomized as CSF amyloid abnormal $\leq$813 pg/mL and amyloid normal >813 pg/mL.[32]

For a subset (n=69, 28%), amyloid PET was available. Subjects were scanned with [18F]florbetaben (n=33), [18F]florbetapir (n=20), [18F]flutemetamol (n=6) or [11C]pittsburgh compound-B (PiB; n=10) radiotracer. Tracers were infused trough a venous cannula. [18F]florbetapir and [11C]PIB scans were acquired through 90 minutes dynamic scanning (respectively PET/CT Ingenuity TF or

Gemini TF, Philips Medical Systems, Best, The Netherlands and ECAT EXACT HR+ scanner, Siemens/CTI, Knoxville, TN) simultaneously starting with tracer injection using a Medrad infusion system (approximately 370MBq [18F] florbetapir, 351MBq [11C]PiB). [18F]florbetaben and [18F]flutemetamol scans were acquired through 20 minutes static PET scanning (respectively PET/ MR and Gemini TF-64 PET/CT scanner, Philips Medical Systems, Best, The Netherlands) starting 90 minutes after tracer injection (approximately 250MBq [18F]florbetaben, 180MBq [18F]flutemetamol). PET scans were visually read and dichotomously scored as either amyloid abnormal or normal by an experienced nuclear medicine physician (BvB).

**Plasma analyses**
EDTA plasma was obtained through venipuncture. After centrifugation at 1800 x g, EDTA plasma was aliquoted in 0.5-mL polypropylene tubes and stored at -80 °C in the VUmc Biobank. Samples were shortly thawed at room temperature and centrifuged at 14.000 x g prior to analyses, to prevent any sample debris from interfering in measurement. Plasma levels of Abeta40, Abeta42 and t-tau were measured simultaneously using the commercially available SIMOA Human Neurology 3-Plex A assay kit (Quanterix, Lexington, USA) on-board of the automated SIMOA HD-1 analyzer (Quanterix, Lexington, USA).18 Manufacturer's instructions were followed including 1:4 automated on-board automated sample dilution. All samples were analyzed in duplicates, randomly divided over two runs that were performed on two consecutive days. Research staff was well-trained for the analytical procedure.

The triplex assay was in-house analytically validated prior to use according to standardized international protocols.33 Abeta40 and Abeta42 gave good average inter-assay variation (Abeta40: 7.4% coefficient of variation (CV), Abeta42: 8.7%CV). Inter-assay variation was higher for t-tau (22.2%CV), caused by poor repeatability of a validation sample with a low tau concentration (only 1.25 pg/ml on average). All patient samples showed values above our in-house quantified lower limit of quantification (LLOQ; Abeta40: 0.16 pg/mL, Abeta42: 0.34 pg/mL, t-tau: 0.42 pg/mL), except for n=10 Tau measurements. Average intra-assay variation of duplicate measurements was well below the accepted cut-off of 20%CV (Abeta40: 3.1%CV, Abeta42: 3.9%CV, t-tau: 5.8%CV). t-tau measurements below LLOQ were assigned the measured concentration, as in our opinion this is more accurate than either assigning 0 (under-estimation) or assigning the LLOQ value (over-estimation). Two t-tau values had an intra-assay %CV >20. Upon repetition of measurement the measured t-tau concentration was very alike, and therefore it was decided to use the initial result. Excluding these 12 t-tau measurements did not alter statistical outcomes.

**APOE genotyping**
Genomic DNA was isolated from EDTA blood. Using PCR technique DNA was amplified and subsequently analyzed using QIAxcel DNA Fast Analysis kit (Qiagen, Venlo, The Netherlands) to establish size, and sanger sequenced on

the ABI130XL to determine Apolipoprotein E (APOE) genotype. One or two APOE e4 alleles classified subjects as APOE e4 carriers, whereas no e4 allele classified subjects as non-carriers. APOE e4 carriership was available for 235 (95%) of our subjects.

**Statistical analysis**
Statistical analysis was performed using SPSS for Windows, version 22 (IBM). $P < 0.05$ was considered statistically significant. Plasma Abeta42 and Abeta40 were used as single variables as well as in the ratio Abeta42/Abeta40 multiplied by 1000. When biomarker data were skewed, natural log transformation was performed prior to correlation and regression analyses (applied for variables: plasma t-tau, plasma ratio Abeta42/Abeta40, CSF tau, CSF pTau181). Prior to logistic regression analyses and COX proportional hazards analyses, plasma Abeta40, Abeta42 and natural log transformed Abeta42/Abeta40*1000 and tau data were inverted and transformed to Z-scores so that lower levels imply higher risk and effect sizes are comparable between markers.
Baseline demographics and clinical characteristics were compared using t-tests, Mann-Whitney U tests and Chi square tests as appropriate. CSF and plasma biomarker levels were additionally compared using age and sex corrected univariate analyses of variance. Associations of plasma biomarker levels and CSF biomarker levels were assessed using Pearson's correlation analyses and visualized in scatterplots constructed using R version 3.4.2. The association of plasma biomarkers with CSF-based and PET-based abnormal amyloid status were assessed using logistic regression analysis followed by receiver operating characteristic (ROC) curve analyses. Predicted values of binary logistic regression models were used to combine variables in ROC analysis. To evaluate the potential of the plasma Abeta42/Abeta40 ratio to identify CSF and PET abnormal amyloid status, the coordinates of the corresponding ROC curve were used to establish the Youden's cut off (i.e. maximal sum of sensitivity and specificity). For visualization purposes, we applied the sensitivity and specificity levels of the Youden's cut-off to calculate how many individuals we would need to screen in total with the blood test to obtain 100 CSF or PET amyloid abnormal subjects. To evaluate the potential of the multivariable model plasma Abeta42/Abeta40 ratio combined with age and APOE e4 carriership to identify CSF amyloid abnormal subjects, heat maps were constructed by filling out the logistic regression formula. Finally, we assessed the association of plasma markers with risk of clinical progression to MCI or dementia using COX proportional hazard models, both unadjusted and adjusted for age and sex. This analysis was repeated excluding subjects that progressed to non-AD dementia. For visualization, Kaplan Meier survival curves were plotted for clinical progression to MCI or AD dementia with separate lines for low, middle and high baseline plasma levels of Abeta42 alone and of Abeta42/Abeta40 ratio (data divided in tertiles).

## RESULTS

### Demographic and clinical characteristics

At baseline, the 248 subjects with SCD were on average 61±9 years old, 42% was female and mini-mental state examination (MMSE) was 28±2. Based on CSF, 57 (23%) subjects had an abnormal amyloid status. After an average follow-up of 3±2 years, 35 (14%) subjects showed clinical progression (Table 1). Of the progressors, 23 to progressed to MCI, 4 to AD dementia and 8 to non-AD dementia (4 to frontotemporal dementia, 1 to vascular dementia, 3 to other types of dementia).

Comparing CSF-based amyloid abnormal to amyloid normal subjects, subjects with an abnormal CSF amyloid status were on average older, more frequently female, had a lower MMSE score and were more frequently APOE e4 carrier. CSF-based amyloid abnormal subjects progressed more often to MCI or dementia ($p<0.05$). Also, CSF t-tau and CSF pTau181 levels were higher in subjects with an abnormal CSF amyloid status compared to subjects with a normal amyloid status (Table 1).

Adjusted for age and sex, plasma Abeta42 alone and plasma Abeta42/Abeta40 ratio were lower in subjects with an abnormal CSF amyloid status compared to subjects with a normal CSF amyloid status (both $p<0.01$, Table 1). Plasma Abeta40 and plasma t-tau did not differ between groups.

### Correlations of plasma and CSF markers

All plasma measures Abeta40, Abeta42 and t-tau were positively correlated with each other (all $r>0.25$; $p<0.001$; Table 2; Fig 1 A, Fig 1 B). Plasma Abeta42 and plasma Abeta42/Abeta40 ratio were positively associated with CSF Abeta42 levels (Abeta42; $r=0.18$, Abeta42/Abeta40 ratio $r=0.38$; both $p<0.001$; Fig 1 C, Fig 1 D), and negatively associated with CSF t-tau and CSF pTau181 (all: $r<-0.23$; $p<0.001$; Fig 1 E, Fig 1 F). On visual inspection, plasma Abeta42/Abeta40 ratio had the strongest correlations with all CSF biomarkers. There were no associations between plasma Abeta40 or plasma t-tau and any of the CSF biomarkers (Fig 1 G, Fig 1 H).

### Plasma markers as predictors of CSF amyloid status

Using logistic regression analysis, we found a positive association of plasma Abeta42/Abeta40 ratio (odds ratio (OR)=3.15 (95% CI: 2.10-4.74)) and of plasma Abeta42 (OR=1.74 (95% CI: 1.24-2.44)) with CSF-based abnormal amyloid status. After adjustment for age and APOE e4 carriership, the associations remained significant (Abeta42/Abeta40 ratio: OR=2.35 (95% CI: 1.53-3.61); Abeta42: OR=1.94 (95%CI: 1.31-2.86)). There was no association between plasma Abeta40 alone or plasma t-tau and CSF amyloid status.

ROC analyses (Fig 2) revealed an area under the curve (AUC) of 77% (95% CI: 69 – 84%) for the plasma Abeta42/Abeta40 ratio and for plasma Abeta42 alone 66% (95% CI: 58 – 74%). Youden's cut-off of plasma Abeta42/Abeta40 ratio was 45 and yielded a sensitivity of 76% and specificity of 75%. As an example, based on our cohort, we would need to perform 434 lumbar punctures to obtain
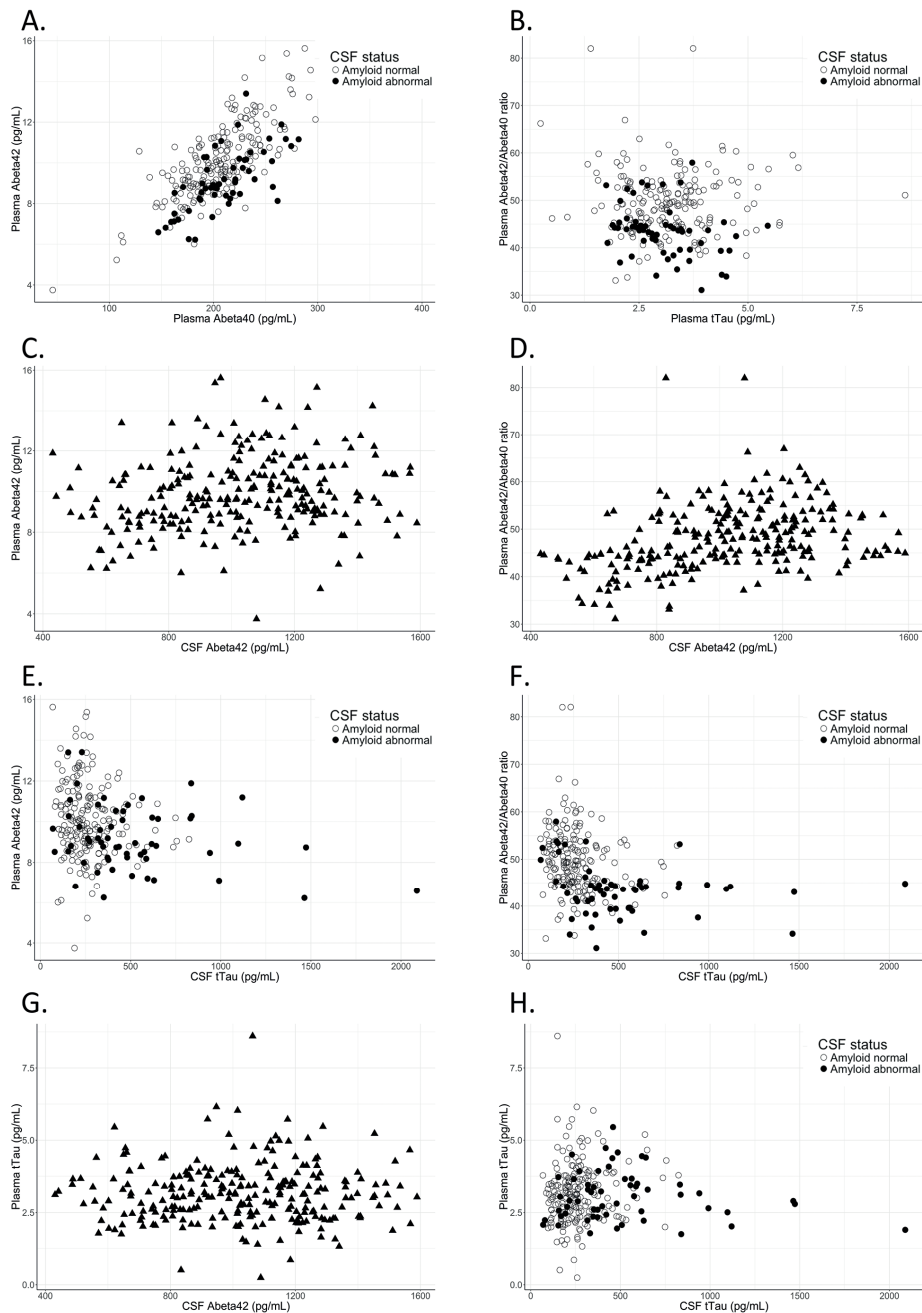
Figure 1. Scatterplots of plasma and CSF markers. Scatterplots presenting the correlation of the plasma marker concentrations (A., B.) and the correlation of plasma marker concentrations with CSF marker concentrations (C. – H.). Triangles = Total study population; Open circles = Subjects with normal CSF Amyloid status ie.e CSF Abeta42 concentration > 813 pg/mL; Closed circles = Subjects with abnormal CSF Amyloid status i.e. CSF Abeta42 concentration ≤ 813 pg/mL.
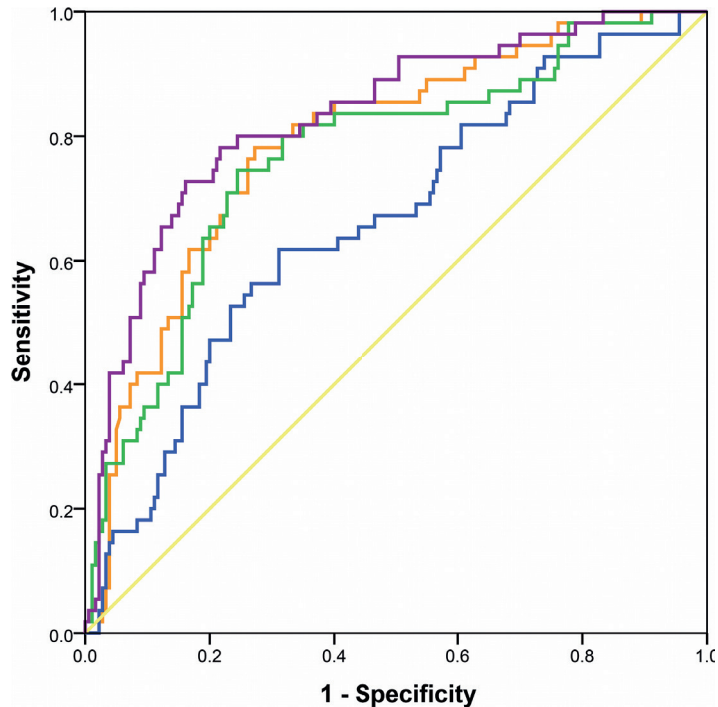
Figure 2. ROC curves predicting CSF-based amyloid abnormality based on plasma Abeta42, Plasma ratio Abeta42/Abeta40 and multivariable models including age and APOE e4 status. Pink = Plasma Abeta42/Abeta40 ratio, APOE e4 carriership and age; Orange = APOE e4 carriership and age; Green = Plasma Abeta42/Abeta40 ratio; Blue = Plasma Abeta42; Yellow = 50%-reference line.

100 subjects with abnormal CSF amyloid status. When applying the Youden's cut-off of the plasma Abeta42/Abeta40 ratio, the number of lumbar punctures would be reduced by 51% (Fig 3).

When combining plasma Abeta42/Abeta40 ratio with age and APOE e4 carriership in a multivariable model, discrimination became good with an AUC of 83% (95% CI: 77%-89%).

Subsequently we used the linear predictor formula of this model to construct heat maps that visualize the probabilities (%) of having an abnormal CSF amyloid status based on age and plasma Abeta42/Abeta40 ratio after stratification for APOE e4 carriership (Fig 4). For example, an APOE e4 carrier of 70 years old with a plasma ratio of 35 would have a probability of 81% to be CSF amyloid abnormal (i.e. 123 lumbar punctures needed to obtain 100 CSF-based amyloid abnormal subjects). By contrast, with this same plasma ratio of 35, the probability of a 70-year old non-APOE e4 carrier to be CSF amyloid abnormal is 57% (i.e. 175 lumbar punctures needed to obtain 100 CSF-based amyloid abnormal subjects), and would be 72% with a plasma ratio of 30 (i.e. 138 lumbar punctures needed to obtain 100 CSF-based amyloid abnormal subjects). This illustrates how such a tool could help in pre-screening for abnormal amyloid status.

A.



Identifying CSF amyloid abnormality

n=434

n=100

Lumbar puncture

B.



Identifying CSF amyloid abnormality

n=572

n=220

n=100

Plasma pre-screening

Lumbar puncture
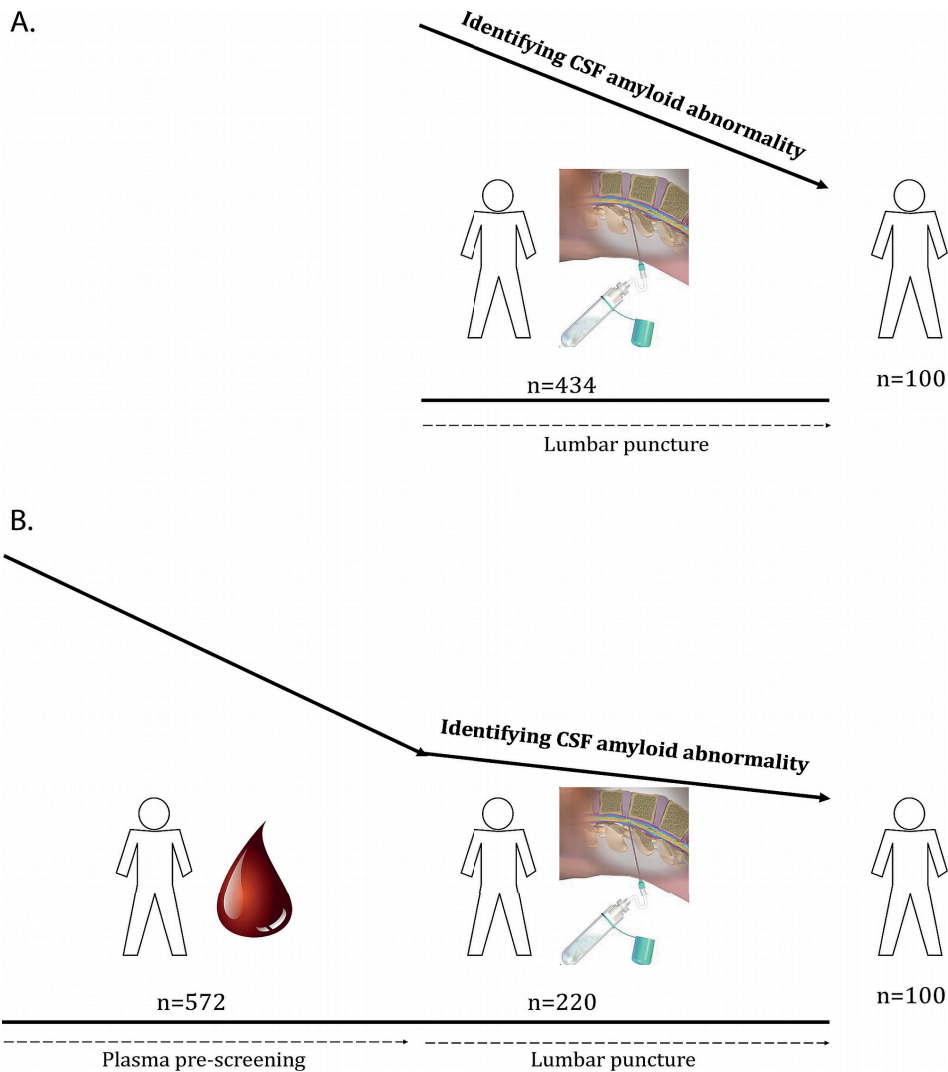
Figure 3. Visualization of the pre-screening potential using the Youden's cut-off for plasma Abeta42/Abeta40 ratio to obtain 100 CSF-amyloid abnormal subjects. For visualization of pre-screening potential in a two-step diagnostics process, prevalence of CSF-amyloid abnormality in our cohort (A.) and the Youden's cut-off of the plasma Abeta42/Abeta40 ratio in our cohort extracted from the ROC coordinates table (B.: cut-off = 45, sensitivity=76%, specificity=75%) were applied. Numbers were extrapolated so that a hypothetical total of 100 CSF-amyloid abnormal subjects would be identified. Plasma Abeta42/Abeta40 ratio was multiplied by 1000 prior to ROC analysis. CSF = cerebrospinal fluid.
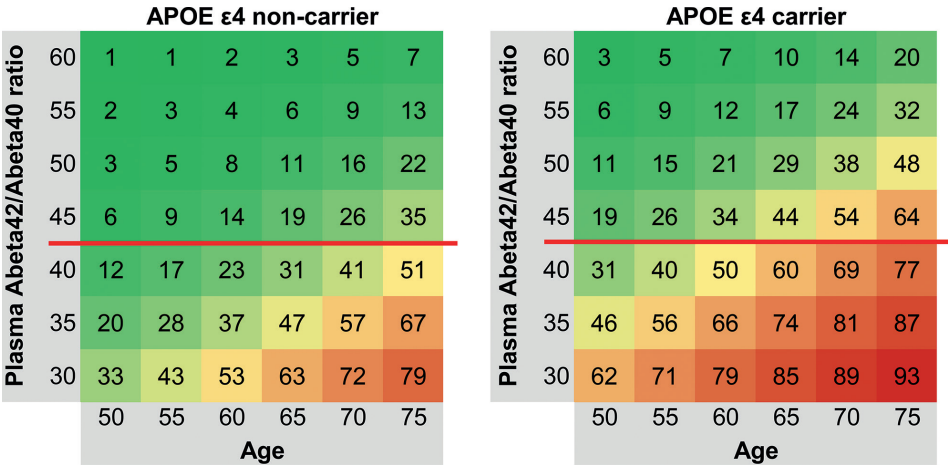
Figure 4. Heat maps showing predicted probability of being CSF-amyloid abnormal based on plasma Abeta42/Abeta40 ratio and age when stratified for APOE e4 carriership. Probabilities are presented in %. The red line indicates the Youden's cut-off of plasma Abeta42/Abeta40 ratio. Plasma Abeta42/Abeta40 ratio was multiplied by 1000 prior to analysis. Heat maps were constructed using logistic regression predictor formula with constant=-0.879 and betas (B) B(age)=0.082, B(plasma Abeta42/Abeta40 ratio =-0.131 and B(APOE e4 carriership)=1.202. Age and plasma ratios were entered as continuous variables, APOE e4 carriership as dichotomous variable with 0=non-carrier and 1=carrier. Abeta=amyloid beta, APOE = Apolipoprotein E.

## Plasma markers as predictors of PET amyloid status

For a subset of 69 subjects, amyloid PET was available. Of these, 23 (33%) were amyloid abnormal based on PET imaging. Subjects with abnormal amyloid PET scans had lower plasma Abeta42 compared to subjects with normal amyloid PET scans (uncorrected p=0.018) and tended to have lower plasma Abeta42/Abeta40 ratio (p=0.057). Plasma Abeta40 and plasma t-tau did not differ between groups.

Assessing the predictive accuracy of plasma amyloid to discriminate subjects with an abnormal amyloid PET scan from subjects with a normal amyloid PET scan we found an AUC of 66% (95% CI: 53%-79%) for plasma Abeta42 alone and 68% (95% CI: 55-82%) for the plasma Abeta42/Abeta40 ratio. In the multivariable model including age, APOE e4 status and plasma Abeta42/Abeta40 ratio the AUC was 79% (95% CI: 67%-91%). Youden's cut-off of plasma Abeta42/Abeta40 ratio was 44 and yielded a sensitivity of 70% and specificity of 78%. As an example, in our cohort 303 PET scans should be performed to obtain 100 subject with an abnormal amyloid PET scan. Applying the Youden's cut-off of the plasma Abeta42/Abeta40 ratio first, the number of PET scans would be reduced by 54% (i.e. 431 blood tests result in forwarding 163 individuals to PET scanning of which 100 will show PET amyloid abnormality).

## Plasma markers as predictors of clinical progression

Finally, we assessed the predictive value of plasma markers for clinical progression. Baseline plasma Abeta42/Abeta40 ratio was lower in SCD subjects with clinical progression to MCI or dementia compared to those who remained

stable during the time of study (p=0.002). This decrease lost significance after adjusting for age and sex (p=0.09). Plasma Abeta42 and Abeta40 alone, and plasma t-tau did not differ between groups.

COX proportional hazards analyses showed an association between lower plasma Abeta42/Abeta40 ratio and increased risk of clinical progression to MCI or dementia (hazard ratio (HR)=2.03 (95% CI: 1.43-2.88)), which remained significant after correcting for age and sex (HR=1.67 (95%CI: 1.15-2.44)). Plasma Abeta42, Abeta40 and t-tau were not associated with risk of clinical progression to MCI or dementia. Excluding subjects that progressed to non-AD dementia, revealed an association between lower baseline plasma Abeta42 alone and Abeta42/Abeta40 ratio and increased risk of clinical progression to MCI or AD (Abeta42: HR=1.74 (95%CI: 1.19-2.56), Abeta42/Abeta40 ratio: HR=2.31 (95% CI: 1.55-3.43) (Fig 5). Associations remained significant after correcting for age and sex (Abeta42: HR=1.68 (95%CI: 1.09-2.60), Abeta42/Abeta40 ratio: HR=1.85 (95%CI: 1.21-2.83)). Plasma Abeta40 and t-tau were not associated with risk of clinical progression to MCI or AD.



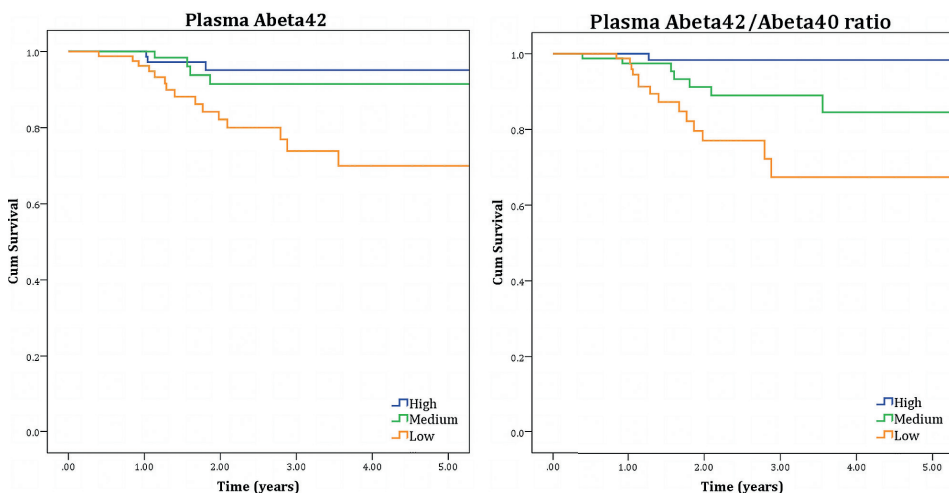Figure 5. Survival curves of baseline plasma Abeta42 and plasma Abeta42/Abeta40 ratio to predict clinical disease progression to MCI or AD dementia. Kaplan Meier survival analysis graphically presenting cognitive decline to MCI or AD dementia upon follow-up when having low (orange), medium (green) or high (blue) baseline plasma Abeta42 (left) or plasma Abeta42/Abeta40 ratio (right). Abeta=amyloid beta.

## DISCUSSION

In the present study we found that plasma Abeta42/Abeta40 ratio has potential as pre-screener to identify the earliest Alzheimer's pathological changes of the AD continuum in cognitively normal individuals with SCD. Combining the plasma Abeta42/Abeta40 ratio with age and APOE e4 yielded an accuracy over 80%. This suggests a future where pre-screening based on a blood test would allow a reduced need of invasive or expensive methods measuring amyloid such as lumbar puncture or PET scanning. In addition, lower plasma Abeta42/Abeta40 ratio was associated with a two-fold increased risk of clinical progression to MCI or dementia.

Plasma Abeta42/Abeta40 ratio was lower in CSF amyloid abnormal individuals compared to amyloid normal individuals and using this ratio we could identify CSF-based amyloid abnormality in our population with an accuracy of 77%. By extrapolating our results, we showed that when applying the optimal plasma Abeta42/Abeta40 ratio cut-off, we could reduce the number of individuals who would need to undergo lumbar puncture by more than half, when first pre-screening with this blood test. Although in our cohort Abeta42/Abeta40 ratio was more strongly associated with CSF amyloid status than with PET amyloid status, the pre-screening effectivity was comparable. We here chose a cut-off maximizing the sum of sensitivity and specificity, which fits with the goal of pre-screening for clinical trial selection. In this context, the impact of missing an amyloid abnormal individual is not very high. The major aim is here to keep costs and invasiveness of screening as low as possible. An alternative goal could be to improve diagnosis of dementia, by applying pre-screening in a general practitioner setting. In such a context, cut-offs should be selected favoring sensitivity, as one would not want to miss any diagnosis. We found that the accuracy increased when we additionally included age and APOEe4 carriership. This shows that a blood marker may have great value in combination with a set of simple additional variables. Adding a cognitive screening tool like MMSE or MOCA, or a larger panel of blood markers might be a promising path to increase both sensitivity and specificity of a pre-screening tool.

Our findings expand on recent findings from other groups that focused on plasma Abeta42 and Abeta40 as putative blood-biomarkers for Alzheimer's pathology.[14-16, 34] With sophisticated but laborious immunoprecipitation and mass spectrometry techniques, two groups showed somewhat higher accuracy of plasma amyloid in predicting amyloid status compared to the accuracy reported in the current study.[15, 16] The complicated nature of their measurement methods however, precludes immediate translation to a clinical setting. Two other studies used automated techniques[14, 34] of which one study used the same analytical platform for plasma analysis as we did.[14] Both studies showed comparable findings as the current study. All former studies compared patients across the spectrum from severe disease to healthy controls, which maximizes the contrast between groups. We deliberately chose a cognitively normal sample

with SCD, which renders achieving high accuracy more challenging. In our view, cognitively normal individuals that present at memory clinics is the target group where a plasma marker should show added benefit. Such benefit in daily practice could only be feasible with an easy to use method, hence our decision to use a straightforward automated analytical technique that would allow large scale measurement of plasma markers on a routine daily basis. Despite having included cognitively normal subjects only in our study, we found a reasonable accuracy to identify Alzheimer's pathophysiology. This is a great leap forward compared to the former generation plasma amyloid analysis methods.12 Our results show that a blood-marker for Abeta becomes feasible, both in a trial setting where increasingly individuals with the earliest AD pathological changes are recruited, but also in a clinical, e.g. primary care setting, to facilitate the diagnostic process.

Plasma t-tau was not altered in the CSF amyloid abnormal group compared to the amyloid normal group. Moreover, plasma t-tau levels were neither correlated with CSF t-tau nor CSF pTau181 levels. Former studies have shown diagnostic value of plasma t-tau, but only at the stage of full-blown dementia.35-39 Thus far, no studies focused on non-demented individuals only. As we sought for differences in this non-demented group, effect size was probably too small to be captured using the current method. By contrast, CSF t-tau and pTau levels in our sample were already altered in CSF amyloid abnormal subjects compared to amyloid normal subjects, suggesting that the technical sensitivity of the plasma t-tau assay used is still insufficient. This reasoning is also supported by the results of our in-house assay validation in which it was shown that the t-tau plasma analysis was performing least well compared to the analysis of the other two markers Abeta42 and Abeta40. Alternatively, it might be that plasma t-tau levels reflect AD pathology to a lesser extent38 than t-tau levels in CSF do. It might be more effective to measure specific tau isoforms in plasma, such as plasma pTau181.39 Combining t-tau with neurodegeneration biomarkers, e.g. neurofilament light,40 might be another promising alternative to increase diagnostic utility.

Some SCD subjects may harbor very early AD pathological changes22 and when comparing an SCD population to a normal aging population they have been found more likely to show clinically progression.41 We found that lower plasma Abeta42/Abeta40 ratio is associated with an increased risk to develop MCI or dementia. In CSF it also found that low CSF Abeta42 concentrations increase the risk of cognitive decline6 and clinical disease progression.42 Although the hazard ratio for clinical progression of the plasma Abeta42/Abeta40 ratio is lower compared to CSF, the finding of the present study shows clinical validity of the plasma measure.

Among the potential limitations of our study is the fact that we had PET data available for only a small number of individuals, obtained with four different tracers, precluding firm conclusions with respect to PET as outcome measure.

Secondly, external validation in an independent cohort should be performed to confirm our findings. Third, we tested our measure in a cohort of SCD individuals and therefore cannot easily translate our findings to the normal aging population. However, we believe that this makes the findings of the current study truly translational to clinical research practice. It has been shown that the presence of subjective memory complaints in itself already represents a higher risk of having high amyloid burden in the brain,43 making this group particularly interesting for clinical trial participant screening and thus benefit from the pre-screening findings we presented here. Other strengths of our study are that our study cohort is well-defined and follow-up including repeated plasma sampling is still ongoing leaving the opportunity to confirm our longitudinal findings in future.

In conclusion, our results strongly suggest that the plasma Abeta42/Abeta40 ratio, measured with an easy to implement, fully automated platform, could serve as a pre-screener, particularly when combined with age and APOE e4 carriership. These results suggest a future where a blood biomarker is applied as pre-screener to pre-select patients for further selection procedure for clinical trials, or for referral to a memory clinic.

**Table 1.** Demographic features of the study population of non-demented elderly (n=403)

| | All | APOE e4 negative | APOE e4 positive | P | SCD | MCI | P |
|---|---|---|---|---|---|---|---|
| N | 403 | 203 | 200 | | 191 | 212 | |
| Age, years | 64.0±9.1 | 63.3±9.5 | 64.8±8.6 | .101 | 60.5±8.8 | 67.2±8.2 | .000 |
| Gender, female | 166 (41%) | 73(36%) | 93(47%) | .034 | 75 (39%) | 91 (43%) | .479 |
| MMSE | 27.3±2.2 | 27.5±1.1 | 27.2±2.3 | .087 | 28.2±1.6 | 26.5±2.4 | .000 |
| APOE e4 positive | 200 (50%) | | | | 74 (39%) | 126 (63%) | .000 |
| APOE e4 allele frequency (0/1/2) | 203/142/58 | | | | 117/58/16 | 86/84/42 | .000 |
| CSF Aβ42, ng/L | 728±281 | 843±268 | 611±243 | .000 | 841±248 | 626±270 | .000 |
| CSF Tau, ng/L | 407±318 | 340±245 | 475±365 | .000 | 289±203 | 513±362 | .000 |
| CSF ApoE, mg/L | 3.5±1.5 | 3.1±1.4 | 3.9±3.1 | .000 | 3.3±1.4 | 3.7±1.5 | .000 |
| CSF clusterin, mg/L | 9.1±3.4 | 9.2±3.6 | 9.1±3.1 | .901 | 2.1±0.4 | 2.2±0.4 | .181 |
| CSF ApoA1, mg/L | 3.7±3.3 | 3.7±4.3 | 3.6±2.0 | .585 | 3.7±4.4 | 3.6±1.9 | .448 |

Demographic features of 403 non-demented participants with Subjective Cognitive Decline (n=191) or Mild Cognitive Impairment (n=212). Data are presented as mean ± standard deviation or n (%). T-tests and Chi-squared tests were used to assess differences between APOE e4 carriers vs. non-carriers, and between diagnoses (SCD vs. MCI). Aβ42 = amyloid beta1-42, ApoA1 = apolipoprotein A1, ApoE = apolipoprotein E, CSF = cerebrospinal fluid, MCI = mild cognitive impairment, MMSE = Mini Mental State Examination, SCD = subjective cognitive decline.

**Table 2.** Linear regression analyses between CSF analytes and APOE e4 allele frequency in non-demented elderly (n=403)

| Variable | Group | APOE e4 allele frequency (1/2/3/) | CSF Aβ$_{1-42}$ | CSF tau | CSF ApoE | CSF ApoJ (Clu) | Age |
|---|---|---|---|---|---|---|---|
| CSF Aβ$_{1-42}$, | All | -.62, p=.000 | | | | | -.32, p=.000 |
| | SCD | -.42, p=.000 | | | | | |
| | MCI | -.65, p=.000 | | | | | |
| CSF tau | All | .31, p=.000 | -.30, p=.000 | | | | .39, p=.000 |
| | SCD | .11, p=.161 | -.07, p=.255 | | | | |
| | MCI | .32, p=.000 | -.32, p=.000 | | | | |
| CSF ApoE | All | .41, p=.000 | -.05, p=.363 | .49, p=.000 | | | .18, p=.000 |
| | SCD | .37, p=.001 | -.02, p=.805 | .65, p=.000 | | | |
| | MCI | .41, p=.000 | -.03, p=.955 | .44, p=.000 | | | |
| CSF ApoJ (clusterin) | All | -.03, p=.670 | .04, p=.434 | .32, p=.000 | .42, p=.000 | | .26, p=.000 |
| | SCD | -.15, p=.152 | -.06, p=.511 | .44, p=.000 | .38, p=.000 | | |
| | MCI | .06, p=.494 | .10, p=.168 | .33, p=.000 | .48, p=.000 | | |
| CSF ApoA1 | All | .06, p=.406 | .04, p=.428 | .07, p=.219 | .08, p=.124 | .37, p=.000 | .14, p=.006 |
| | SCD | .13, p=.285 | .09, p=.323 | -.00, p=.974 | .05, p=.492 | .40, p=.000 | |
| | MCI | .02, p=.831 | .02, p=.804 | .12, p=.073 | .10, p=.104 | .35, p=.000 | |

Overview of associations between CSF analytes and APOE e4 allele frequency in non-demented elderly (n=403), subsequently stratified for diagnosis (SCD and MCI). All linear regression analyses were adjusted for age and gender, results are displayed as Beta with P-value. We also show unadjusted associations between age and CSF analytes. Aβ1-42 = amyloid beta 1-42, Apo = apolipoprotein, Clu = clusterin, SCD = subjective cognitive decline, MCI = mild cognitive impairment.

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# CHAPTER 8
Summary and general discussion
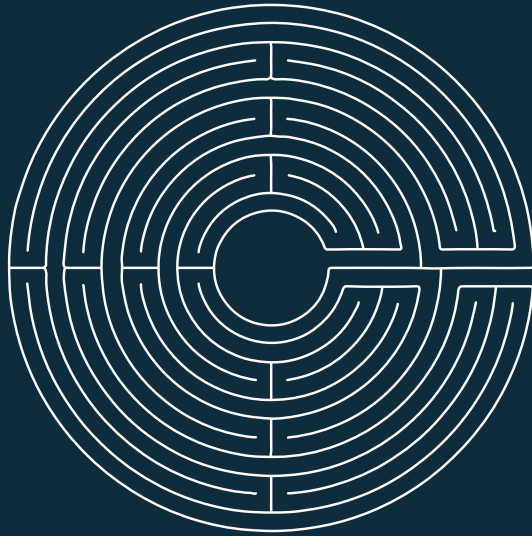
### SUMMARY OF FINDINGS

The aim of this thesis was to investigate the concept of SCD and biomarkers of AD in the preclinical stage of the disease. First we described characteristics of individuals with SCD in the Subjective Cognitive ImpairmENt Cohort (SCIENCe). Second, we investigated risk factors of clinical progression from SCD to Mild Cognitive Impairment (MCI) or dementia. Finally, we evaluated early biomarkers of AD, and biomarkers of future clinical progression to dementia in initially non-demented elderly with SCD and MCI.

### Part I: Subjective cognitive decline: characteristics

In **chapter 2** we evaluated characteristics of individuals with SCD in the Subjective Cognitive Impairment Cohort (SCIENCe), which we set up in the Alzheimer Center Amsterdam in 2014. In a cross-sectional baseline evaluation of the first 150 participants with SCD, we observed a heterogeneous nature of complaints. Around 25% of cognitive normal individuals had evidence of preclinical AD, defined as CSF Amyloid-beta1-42(Abeta42) levels below cut-off and/or Amyloid-positivity visualized using amyloid positron emission tomography (PET) scans. Although individuals with apparent underlying psychiatric nature of cognitive complaints were not included in the cohort, roughly one third of participants had subthreshold psychiatric symptoms evaluated with questionnaires, leaving the largest part of participants with complaints of undetermined origin. In this paper we also investigated the SCD-plus criteria, which have been proposed by the SCD-I working group as possible indicators of preclinical AD [1]. We found that SCD-plus criteria 'age above 60' and 'Apolipoprotein E (APOE) genotype' were cross-sectionally associated with an increased risk of preclinical AD.

### Part II: Subjective cognitive decline: risk factors of clinical progression

Evaluating SCD in a broader perspective, we assessed incidence rates of dementia in individuals with normal cognition and cognitive complaints and in controls without complaints, in a large international collaborative study including over four thousand cognitive normal participants (**chapter 3**). We combined longitudinal data from several cohort studies from Europe, Australia and the United States. Incidence rates of dementia were higher in individuals with SCD compared to cognitively normal controls without cognitive complaints. We observed these findings both for AD and non-AD dementia (e.g. vascular dementia, frontotemporal dementia). Adjusted Cox proportional hazards models showed that risk factors for progression to dementia were higher age, lower MMSE and APOE e4 genotype. Recruitment setting (memory clinic setting, as opposed to community cohort setting) contributed substantially to the risk of clinical progression to dementia.

In **chapter 4** we assessed individual risk estimates of clinical progression in individuals with SCD in a memory clinic setting, using MRI and CSF data. We used individual patient data such as CSF Abeta42, CSF tau and MRI measures,

both volumetric data and visual rating scales. Based on demographics only, five and three-year prognostic models were generated, using Cox regression models. While normal CSF Abeta42 and tau values decreased probabilities of progression to dementia and abnormal CSF values increased risks, individual MRI values did not contribute to the risk of progression. Results were validated externally in ADNI and a European dataset.

**Part III: Biomarkers of Alzheimer's Disease in pre-dementia stages**

In **chapter 5** we investigated associations between high density lipoprotein (HDL) -cholesterol transporter apolipoprotein A1 (ApoA1) and the risk of clinical progression to MCI and dementia. We evaluated associations in a memory clinic sample of individuals with SCD and MCI with available CSF and plasma. We found that higher levels of ApoA1 in CSF and lower plasma ApoA1 levels were associated with an increased risk of clinical progression, after adjustment for age, gender and MMSE. This risk was particularly strong in APOE e4 allele carriers with SCD. We further evaluated apolipoproteins in **chapter 6**, and cross-sectionally investigated the influence of ApoA1, apolipoprotein E (ApoE) and apolipoprotein J (clusterin) levels in CSF, on associations between APOE genotype and CSF Abeta42 and CSF tau. These associations were evaluated in a memory-clinic based population of non-demented individuals with SCD and MCI, using mediation analysis with linear regression analyses. We found that ApoE and clusterin levels mediated the relation between APOE genotype and CSF tau. Of note, there was no influence of these apolipoproteins on the relation between APOE genotype and CSF Abeta42, suggesting involvement of ApoE and clusterin in Amyloid-independent pathways. Finally, in **chapter 7** amyloid-beta in plasma was measured with the novel SIMOA technique. We describe the added value of the plasma Abeta42/Abeta40 ratio in identifying both abnormal CSF-Abeta42 status and abnormal Amyloid PET status with a sensitivity of 76% and specificity 75%, becoming even more discriminative when age and APOE were added to the model (AUC 83%). This suggests the potential for the plasma Abeta42/Abeta40 ratio as a pre-screening tool to identify preclinical AD in SCD. Also, lower plasma Abeta42/Abeta40 ratio in SCD was associated with a twofold increased risk of clinical progression to MCI or dementia.

## DISCUSSION

In the following paragraphs findings will be integrated and placed in the context of current literature, discussing methodological considerations, clinical perspectives and future directions. This chapter ends with recommendations for future research and final remarks.

### METHODOLOGICAL CONSIDERATIONS
Several methodological themes have tobe considered when interpreting results presented in this thesis. Specific methodological considerations are discussed more in depth in the different chapters of this thesis, in the following paragraphs general reflections are summarized.

### SCD concept
The basis of this thesis was the concept of subjective cognitive decline. SCD refers to the subjective experience of cognitive deterioration, whilst at objective cognitive evaluation neuropsychological tests are normal [1]. Moreover, there are no other underlying factors that could possibly explain the subjective experience of cognitive decline, such as epilepsy, metabolic disorders or a psychiatric diagnosis like a depressive disorder. In the Amsterdam Dementia Cohort all SCD participants were referred to the memory clinic by their general practitioner because of cognitive complaints. Cognitive functioning was defined as normal when standardized neuropsychological tests were within range of normal performance, compared with a Dutch reference population. Our definition of SCD may differ from other cohorts, where SCD may be defined, for example, as cognitively normal individuals answering affirmatively on a questionnaire evaluating the presence of memory complaints (yes/no) (**chapter 3**). Our studies included relatively large samples of SCD participants with extensive phenotyping and longitudinal follow-up, including individuals progressing to dementia. SCD participants were evaluated in a highly standardized way in the Amsterdam Dementia Cohort (ADC) and SCIENCe project [2,3]. Clinical evaluation included repeated neuropsychological testing, and biomarker analyses using MRI-scan, amyloid PET-scan, and blood and cerebrospinal fluid analyses (figure 1, **chapter 2**). Biomarker availability was relatively high compared to other SCD cohorts. When participants were included in the SCIENCe cohort, the research context of the clinical follow-up and systematical collection of biomarkers was emphasized. Results of biomarkers were not disclosed in a standardized fashion in our studies, but in a research setting disclosure of amyloid makers is currently investigated in non-demented individuals in the ADC [4].

When evaluating clinical progression in SCD, we used the clinical diagnosis of MCI and dementia due to AD as outcome measures in some studies. Individuals with MCI are considered to be a heterogeneous group, with various underlying pathologies. In our studies most individuals had amnestic MCI and progressed to AD in a later stage. Also, these individuals had more frequently an AD biomarker

profile, which makes it plausible that AD was the underlying pathology in these patients. Nonetheless, because of different underlying causes the MCI stage may seem a less optimal outcome measure. In **chapter 3** we had the opportunity to evaluate the incidence of dementia in SCD using dementia due to AD as an outcome measure, suggesting the increased risk of progression from SCD to dementia compared to cognitively normal individuals without complaints. The longitudinal data we used in our studies comprised a relatively short period, considering that already twenty to thirty years before a diagnosis of dementia the first changes pathological changes occur [5]. Recruitment and follow up of SCIENCe participants is currently ongoing, and will hopefully provide more insight in factors contributing progression or even protective factors.
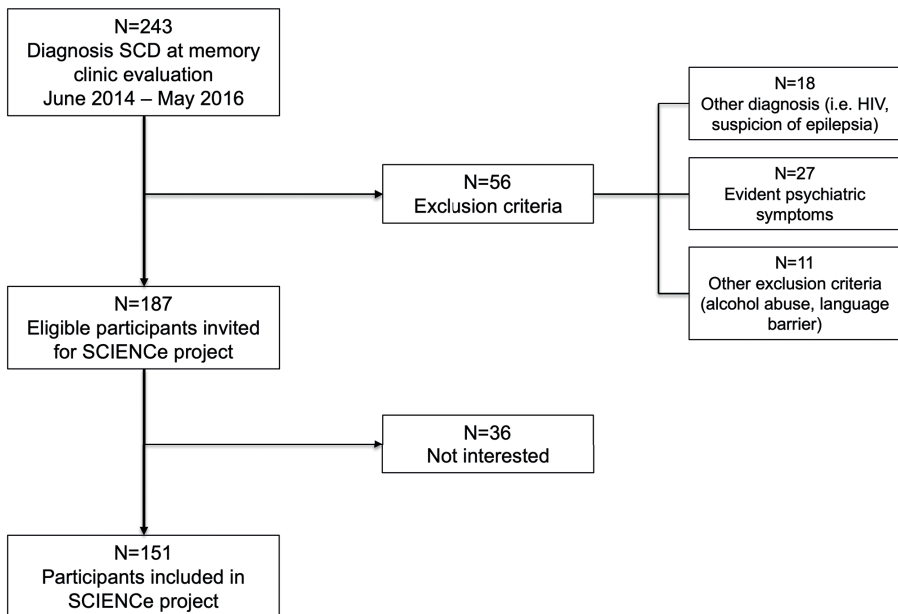


Figure 1. SCIENCe work up (Alz. Res. Therapy, Slot et al., 2018)

A strong aspect of this thesis was the use of different cohorts to evaluate SCD. In our collaborative study on incidence of dementia in SCD (**chapter 3**), we evaluated not only memory-clinic based, but also community based, cohorts from several different countries in Europe, Australia and the USA. In this study we evaluated for the first time such a large sample of individuals with SCD all together (SCD n=2978, compared with n=1391 cognitive normal individuals without complaints). In the SCD research field one is aware of the differences in operationalization of SCD and the possible hampering consequences of these differences. In the collaborative study, initiated at a research meeting of the Alzheimer's Association International Conference, we attempted to overcome these differences and collaborated on an international and even intercontinental level. When we evaluated the risk of clinical progression to

dementia in SCD in this longitudinal study, we found that cohorts from different countries differed substantially on various factors. Because of the differences in demographic factors between cohorts, such as age, MMSE and education, but also less frequently assessed variables such as alcohol intake and family history, we incorporated the influence of center as a putative confounder. We found that center was the variable contributing the largest part to the risk of AD, even after adjusting for other possible confounders. By carefully modeling center and other confounders, we were able to show that SCD participants in memory clinic cohorts had higher rates of progression to dementia than in community based cohorts. The influence of recruitment methods in SCD research has been described before [6–8], and suggests that further evaluation is necessary. To overcome this issue, great efforts have been made by the SCD-I working group to harmonize SCD research by formulating a feasible definition of SCD and suggestions for SCD measurements to be used internationally [1]. Subsequently, the SCD-I working group has made recommendations how to implement the SCD concept in daily (research) practice [9]. To better understand the relation between SCD and the risk of progression to dementia, harmonization of research methods and international collaboration is necessary to be able to generalize results from research to the general population. Further collaborative studies are currently ongoing, and are likely to contribute to a better understanding and execution of the SCD concept in different cohorts and countries, for example the Euro-SCD project and other collaborations by the SCD-Initiative working group [1,10–13].

**Biomarker analyses**
In our studies all CSF and plasma analyses were performed in the Neurochemistry Laboratory of the VU University Medical Center (Amsterdam UMC). CSF and blood collection, and pre-analytical processing and analyses were performed in a standardized way, according to international standards [14,15]. CSF Abeta 42, tau and phosporylated tau-181 were measured using commercial ELISA (Fujirebio Europe, Ghent, Belgium), with good assay quality controls previously validated in our cohort [14]. CSF and plasma apolipoprotein analyses (chapter 6 and 7) were also performed using a commercial ELISA by Mabtech, Nacka Strand Sweden. Quality parameters for these measures were good, but further replication of results is necessary to clinically validate findings. In the years following our apolipoprotein measurements the novel sensitive Single Molecule Array (SIMOA) technology became available [16]. SIMOA brought a novel immunoassay method to evaluate proteins in small levels in for example blood and CSF, opening up possibilities to analyze neuronal markers in blood. We used SIMOA to analyze amyloid-beta 1-40 (Abeta40), Abeta42 and total tau in plasma of individuals with SCD, and found that plasma amyloid-beta holds great promise as a diagnostic biomarker (chapter 8). Findings were in same line with previous studies [17,18]. Further replication of results and extension of other biomarkers in our SCD cohort is currently ongoing.

**CLINICAL IMPLICATIONS**

The SCD concept is undoubtedly relevant for the clinician, since around 20% of patients referred to a memory clinic receive a diagnosis of SCD upon first evaluation [2,19]. In the Amsterdam Dementia Cohort, this is the second most frequent diagnosis, after AD dementia (30%)[2]. Our findings indicate that SCD is not merely a reassuring conclusion after cognitive evaluation, since we observed that individuals with SCD have higher rates of clinical progression to dementia than individuals without cognitive complaints (**chapter 3**). Nonetheless, most individuals with SCD do not progress to dementia. For the clinician it is therefore important to be able to reassure those individuals with low risk of progression, while recognizing those with an increased risk. To distinguish which individuals with SCD have an increased risk of progression is rather challenging in daily practice. We evaluated the prevalence of preclinical AD in SCD in the SCIENCe project in **chapter 2**. Preclinical AD was defined as abnormal CSF Abeta42 and/or abnormal amyloid PET scan. We cross-sectionally observed in SCIENCe that around 25% of patients with available amyloid status had preclinical AD, defined by abnormal CSF Abeta42 values or an abnormal amyloid PET scan (chapter 2). This is similar to somewhat higher than in previous studies, where results were similar to those performed in a memory clinic setting and somewhat higher than in a large studying including information from mostly community cohorts, but also memory clinics [20–23]. The percentage of preclinical AD in our study increased with age. Although we did not make a formal comparison, percentages of amyloid positivity per decade seem somewhat higher in our cohort than in individuals with SCD in a recent large meta-analysis investigating amyloid prevalence in non-demented elderly in a combined sample of memory clinic and community based individuals [22].

In SCD, abnormal Abeta42 values measured by CSF and/or amyloid-PET scans increase the risk of progression to dementia [20,23,24]. Translating preclinical AD results from group level findings to the individual patient is difficult. Hence, several questions rise for the clinician evaluating SCD and preclinical AD. Relevant questions include 'will every amyloid positive patient eventually progress to dementia?' and 'which factors can predict when there will be clinical progression?', but also 'which patients can be reassured that the risk of dementia is low?' and 'which patients might have possible treatment options, if available in the future?'. To facilitate the clinician in managing SCD in daily practice, recent progress has been made in identifying risk factors of clinical progression to dementia. In 2014 the SCD working group introduced the SCD plus criteria as factors that might increase the likelihood of preclinical AD in SCD [1]. These criteria are based on previous research, and include biomarkers such as APOE e4 carriership, but also patient specific features such as predominant self-perceived memory decline and feeling of worse memory performance than others of the same age. In **chapter 2** we evaluated the SCD plus criteria in the Subjective Cognitive Impairment Cohort (SCIENCe), and found that particularly the criteria age>60 and APOE e4 carriership were associated with an increased

risk of preclinical AD (**chapter 2**), which is in line with literature [22,23,25]. Also, in our large collaborative study on the incidence of SCD and risk factors of progression (**chapter 3**), we found that higher age, lower MMSE and APOE carriership gave an increased risk of dementia. The four other SCD-plus criteria we evaluated in SCIENCe (**chapter 2**) were not significantly associated with preclinical AD in our cohort, although there was a trend for an increased risk of preclinical AD when the informant reported significant decline. Previous studies indicated that patients with SCD have an increased risk of progression to MCI or dementia, especially when complaints are reported by both patient and informant [26–31]. The lack of association between the other SCD plus criteria and preclinical AD might be a result of different settings and questionnaires used. The SCD plus criteria need further evaluation and validation. Recent studies indicated that in addition to the report of memory complaints by cognitively normal people, worries about cognitive performance were associated with an increased risk of progression from SCD to dementia and also amyloid-beta load in SCD [32–34]. Worries about cognitive performance might be a useful addition to these SCD plus criteria.

To be able to combine information from different risk factors and biomarkers, recent efforts have been made in the development of individual risk scores of AD for non-demented elderly. In a recent study from our center in patients with MCI, results seemed promising for individual risk estimates based on cognitive, MRI and CSF markers [35]. With a similar approach we assessed individuals risk scores for SCD in our cohort (**chapter 4**). In line with previous literature on group level, we found that abnormal CSF Abeta42 and tau levels contributed to the risk of clinical progression on an individual level [20]. Of note, MRI measures did not significantly contribute to the risk, which might indicate that CSF abnormalities precede structural cortical changes, as previously suggested [5,36]. For the SCD participants in our cohort personalized risk scores based om CSF and MRI markers had particularly strong negative predictive values.

A strong negative predictive value of more commonly used AD biomarkers in SCD provides a helpful tool for the clinician to be able to answer the question proposed above: 'which patient can be reassured that risks of cognitive decline are low?'. Although we observed that cognitive normal individuals with SCD have an increased risk of progressing to AD compared to those without complaints, most individuals with SCD do not progress to AD (**chapter 3**). Regarding possible treatment options, it is therefore not only relevant to identify those at risk of clinical progression, but also those individuals with SCD with a low risk of developing dementia. This, to be able to reassure those with a low risk of dementia, and also since several non-pharmacological treatment options are suggested for the latter group [12]. Non-pharmacological interventions include dietary changes and online lifestyle suggestions, while mindfulness training is currently investigated [37–39].

In **chapter 2** we observed that more than one third of SCD participants in the SCIENCe cohort experienced subthreshold psychiatric symptoms, although

the presence of a formal psychiatric diagnosis was an exclusion criterion for participation in SCIENCe. When there was suspicion of an underlying psychiatric diagnosis, patients were evaluated by a psychiatrist specialized in neuro-cognitive disorders, and excluded when patients met DSM-IV criteria for a diagnosis explaining cognitive complaints. Psychiatric symptoms were evaluated using questionnaires on anxiety, depressive symptoms, features of neuroticism or stress. Psychiatric features have been described as a possible underlying cause of memory complaints, and might provide an alternative explanation for the subjective experience of decline [40–44]. On the other hand, several of these psychiatric features, such as anxiety, neuroticism and distress, have also been associated with preclinical AD [45–51]. We observed the co-occurrence of preclinical AD and subthreshold psychiatric symptoms in 8 of 28 cases. Further assessment of these relations and possible interactions is currently ongoing. Overall, it is important for the clinician to be aware that symptoms of anxiety or depression could be a first sign of imminent AD [48].

## IMLICATIONS FOR FUTURE RESEARCH

### Subjective cognitive decline research

With growing interest in the SCD concept the past years, SCD is investigated in different clinical and research settings all over the world. From our collaborative SCD study (**chapter 3**) we learned that cohorts evaluating SCD differed substantially in demographic features, but also in execution of the diagnosis or concept of SCD. The observation that individuals with SCD in a memory clinic setting have a higher risk of progression to dementia than in community cohorts could not be entirely explained by these substantial center differences in demographics [6,8,52]. Maybe help seeking behavior of the individual itself is also of influence [6,7]. Our findings emphasize the need for further harmonization of the SCD concept between countries and cohorts. Important steps in harmonization of SCD have been taken by the publication of the conceptual framework for research on SCD and preclinical Alzheimer's disease by the SCD-I working group in 2014, and more recently in 2017 the implementation of subjective cognitive decline criteria in research studies [1,9]. For future studies individuals with SCD recruited in a memory clinic setting might provide an enriched population to investigate new therapies. This could have relevance both for disease modifying drugs such as anti-amyloid therapies, since the prevalence of preclinical AD seems somewhat higher in SCD in memory clinic than in community cohorts (**chapter 2 and 3** [22]), and for non-pharmacological lifestyle modification interventions. Several potentially modifiable risk factors of AD have been identified, such as cardiovascular risk factors (i.e. diabetes, hypertension, obesity) and lifestyle habits (i.e. smoking, diet, mental and physical activity) [53,54], but contributing mechanisms remain unknown. Lifestyle interventions to prevent cognitive decline and AD are currently being investigated in non-demented elderly in a randomized controlled set-up [55]. More specifically in SCD, recent efforts have been made to identify preferences of participants in possible lifestyle interventions [12,37].

### Biomarker trends

AD biomarkers in CSF or amyloid PET are more and more widely used in general neurological or geriatric practices. Evaluation of feasibility, cost-effectiveness and patient compliance of CSF and PET in daily practice is currently ongoing in the ABIDE project [56]. During the period in which this thesis has been written, novel techniques for measuring biomarkers have developed rapidly, and techniques became more easily available. When we started the SCIENCe project, Alzheimer pathology could be investigated in vivo using CSF AD biomarkers Abeta42 and tau, and amyloid PET-scans. Meanwhile, novel techniques have been developed, of which the tau PET scan and SIMOA technology for blood biomarkers have had great impact on the field.

CSF Abeta42 and amyloid PET scans have shown to be robust markers of future cognitive decline in preclinical AD [20,23,24]. Another (non-amyloid) actor in the pathogenesis of AD is total tau [57]. CSF tau has been associated with future

decline, and tau PET shows region specific alterations, already in SCD [20,58]. In the SCIENCe project, tau PET scans are currently being investigated and results are to be expected soon. Lumbar puncture to obtain CSF and administration of a radio-active tracer when performing a PET scan are relatively invasive techniques, and less easily available outside memory clinics. Therefore, there is a need for more easily available biomarkers. In search of better accessible biomarkers, Abeta42 in plasma has been evaluated for years. Until recently, results were variable and sometimes conflicting, probably because of pre-analytical variation or lack of sensitivity of methods [17]. With the arrival of more sensitive methods, such as the ultrasensitive SIMOA platform, identification of subtle changes in biomarker profile became available [59]. **In chapter 7** we describe the results of Abeta42 changes measured in blood of individuals with SCD and an increased risk of AD. From previous studies we know that low CSF Abeta42 is associated with an increased risk of progression to AD [20,24]. We found that already in SCD reliable Abeta42 changes could be detected in plasma using the SIMOA method. Findings suggest that the plasma Abeta42/Abeta40 ratio could be a sensitive pre-screening tool, able to identify those non-demented individuals with an increased risk of AD. In the nearby future individuals at risk could be screened in general practice and if needed referred to a memory clinic.

More recent insights suggest a role for amyloid-dependent and amyloid-independent pathways influencing the pathogenesis of AD side by side [60,61]. Amyloid-independent processes associated with AD include the contribution of vascular pathology, alterations in lipid metabolism and immunological processes [62–64]. One of the major risk factors for AD is APOE e4 carriership, encoding for lipid transporter apolipoprotein E [65]. How APOE affects the pathogenesis of AD is still topic of discussion [66]. We observed in **chapter 5 and 6** that in SCD apolipoprotein CSF levels differed between APOE e4 carriers and non-carriers, and alterations in CSF ApoA1 levels were associated with clinical progression in SCD (**chapter 5**). Previously, ApoA1, ApoE and clusterin came up in larger proteomic studies investigating plasma biomarkers of AD [67–69].

In **chapter 6** we found that CSF ApoE and clusterin levels influenced associations between APOE allele frequency and CSF tau, but not Abeta42 (figure 2). Although a dose dependent effect of APOE e4 allele frequency on CSF Abeta42 values has been described previously [70], mechanisms remain to be elucidated. Our findings in **chapter 5 and 6** suggest a role for lipid alterations contributing to the pathogenesis of AD already in pre-dementia stages. A recent large GWAS identified multiple lipid metabolism-related genes associated with an increased risk of AD, independent of APOE [71], and cholesterol esters have been suggested to alter Abeta and tau expression independently [72]. Further replication of alterations in lipid metabolism, more specifically apolipoproteins, using more sensitive methods such as SIMOA should be included when evaluating plasma proteins in search of contributors to the to the pathogenesis of AD. It should be kept in mind that peripheral blood biomarkers, such as apolipoproteins,
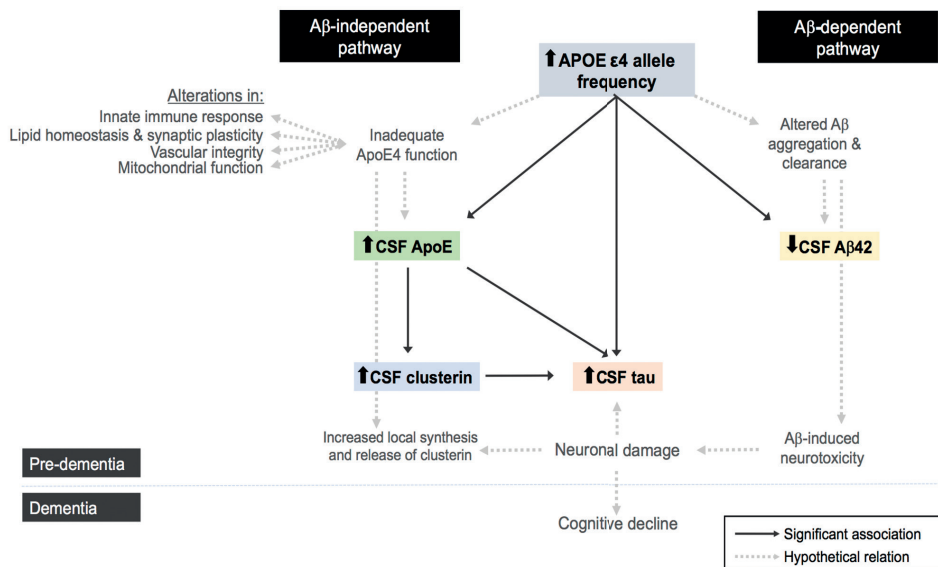
Figure 2. Apolipoproteins mediate associations between APOE genotype and CSF tau (Slot et al., Neurobiology of Aging, 2019)

CSF Abeta42, amyloid PET scan, and even plasma Abeta42 or the Abeta42/Abeta40 ratio, may be reliable risk indicators for future cognitive decline in SCD. Nonetheless, Abeta42 biomarker values do not give information about the stage of disease along the AD continuum. Where to place a non-demented individual with abnormal amyloid markers along the AD continuum is highly challenging. At present, it is not possible to predict whether an individual with SCD has twenty or two years until future significant cognitive decline. This identification is very relevant for the individual and clinician as well, in terms of disease management and, in the future, timing of treatment initiation. Therefore, there has been an increasing focus on prognostic markers to predict future progression and also markers to measure the rate of progression.

Abnormal amyloid, abnormal tau and signs of neurodegeneration have all been shown to increase the risk of future cognitive decline in individuals with SCD [20,24,74–76]. More recent a new research framework has been developed to categorize these biomarkers in the following categories: amyloid beta markers (A), pathologic tau (T) and neurodegeneration (N) [77]. The ATN framework focusses on the continuum of A-T-N biomarkers in different disease stages, and facilitates in identifying different disease processes that co-occur and influence each other. Simultaneously, clinical staging of the AD continuum has been expanded, of which SCD is stage 2 [77]. The concomitant effects of these different ATN biomarkers have not often been evaluated together in SCD. The ATN combination showed to be of help in non-demented elderly to identifying those at risk of progression [78]. The classification system might aid in further understanding of biomarker interaction, and hopefully also temporal ordering or

markers. Then, how many different diagnostic tests are necessary to be able to predict progression using the ATN framework. Nowadays, one needs MRI scans, CSF and also PET-scans to complete information in the ATN model. Ultimately, it would be ideal if there were easily available marker in one matrix, for example measured in blood, giving information about the different ATN categories. One of the novel biomarkers that seems of interest to evaluate neurodegeneration is neurofilament light (Nfl). Recent developments in the measurement of neurofilament light (Nfl) in CSF and plasma, using SIMOA technology, indicate that Nfl is an interesting marker of nearby relevant decline in cognition in pre-dementia stages [79-81]. Although Nfl is a less specific marker for AD, as there are also alterations in other neurodegenerative diseases, as well as for example multiple sclerosis [82], an ongoing study observed that already in non-demented elderly Nfl is altered (Verberk et al., submitted). In the end, it would be ideal to be able to predict the risk of AD in cognitively normal by measuring plasma amyloid values, combined with information about disease stage using (p)tau and Nfl, and other yet to be determined plasma biomarkers informing about the complete ATN framework, including markers of inflammation, synapse loss and lipid alterations.

**CONCLUDING REMARKS**

This thesis endorses the importance of the concept of SCD from both a research and clinical perspective. Our findings indicate that SCD is not merely a benign condition. Although the majority of individuals with SCD do not harbor any neurodegenerative disease, roughly one of four SCD participants in our studies has preclinical AD, and we show not only that progression rates to dementia are higher in SCD than in individuals without cognitive complaints, but also that preclinical AD is associated with future clinical progression. Specific knowledge of underlying causes of cognitive complaints in SCD, such as preclinical AD or for example subthreshold psychiatric features, may aid in determining further steps for the individual with SCD. Not only for individuals with SCD and preclinical AD future treatment options should be considered. Also for individuals with SCD but without preclinical AD, non-pharmacological interventions such as psychoeducation or lifestyle changes may provide a solution for their complaints. Our biomarker findings indicate that CSF and plasma apolipoprotein changes in non-demented APOE carriers, are associated with an increased risk of AD. Also, the plasma Abeta40/42 ratio measured with SIMOA provides a promising prescreening tool in SCD to predict future decline. Further application of the ATN model in pre-dementia AD research, whilst implementing novel biomarkers, will aid in unraveling the different processes contributing to the pathogenesis of AD. Personalized risk scores and early AD screening tools will aid the clinician in determining whether an individual with SCD should be referred to a specialized memory clinic and receive further analysis. Ultimately, the goal is to identify which cognitive normal individuals with a high risk of AD are eligible for treatment. When accurate risk estimates can be provided for the individual, personalized preventive interventions can be initiated.

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# ADDENDUM

ADDENDUM

**NEDERLANDSE SAMENVATTING**

**Dementie en de ziekte van Alzheimer**
Dementie wordt gekenmerkt door een achteruitgang in het denken (cognitief functioneren), bijvoorbeeld geheugenproblemen. Daarbij is een persoon niet meer in staat goed voor zichzelf te zorgen, we noemen dit interferentie in het dagelijks leven. Naast achteruitgang in geheugenfunctie kunnen er ook stoornissen zijn in de taalfunctie, aandacht of oriëntatie, evenals neuropsychiatrische symptomen, zoals depressieve symptomen of gedragsverandering.
Dementie is een groot probleem voor de volksgezondheid en de kosten van de zorg voor mensen met dementie zijn hoog. Onder andere door de vergrijzing van de bevolking neemt het aantal mensen met dementie jaarlijks toe en er wordt geschat dat er in 2030 wereldwijd 65 miljoen mensen met een diagnose dementie zijn.

Dementie kan veroorzaakt worden door verschillende neurodegeneratieve ziekten. De ziekte van Alzheimer is de meest voorkomende oorzaak van dementie, naast bijvoorbeeld vasculaire dementie of frontotemporale dementie. De ziekte van Alzheimer is vernoemd naar dokter Alois Alzheimer. Hij beschreef in 1907 voor het eerst een patiënte met ernstig geheugenverlies, waarbij hij na haar overlijden in de hersenen typische eiwit veranderingen zag. De ziekte van Alzheimer wordt gekenmerkt door ophopingen van het amyloid-beta tússen de hersencellen en ín de hersencellen ontstaan ophopingen van het eiwit tau.
Tot nu toe is er geen effectieve behandeling tegen Alzheimer dementie beschikbaar, ondanks dat er veel verschillende medicijnonderzoeken gericht op het verwijderen van het eiwit amyloid uit de hersenen gedaan zijn.
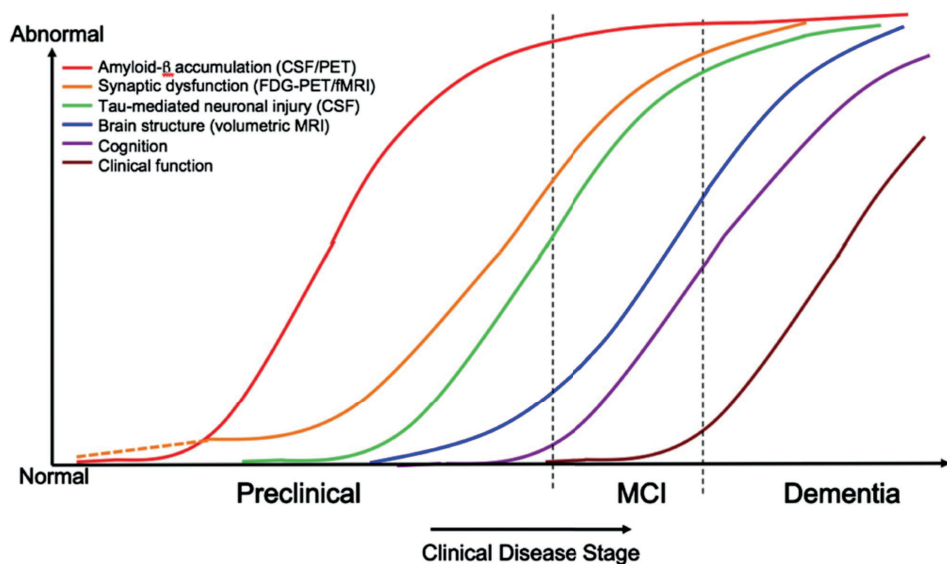
**Biomarkers van de ziekte van Alzheimer**
Een biomarker, of biologische marker, is een indicator van een toestand of ziekte, bijvoorbeeld een eiwit. In de zoektocht naar een behandeling van de ziekte van Alzheimer doet men onderzoek naar de biologische veranderingen (biomarkers) in het lichaam en de hersenen van mensen met dementie. Bij de ziekte van Alzheimer zijn er veranderingen van eiwitten in het hersenvocht, bijvoorbeeld veranderingen in de hoeveelheid amyloid-beta (Abeta), tau en gefosforyleerd tau (p-tau). Met behulp van positon emissie tomografie (PET) scans kan men het Alzheimer eiwit Abeta meten in de hersenen. Op beeldvorming van de hersenen met behulp van een MRI-scan ziet men krimp van de hersenen (atrofie). Recente onderzoeken beschrijven dat, naast veranderingen in het amyloid eiwit, ook vasculaire veranderingen, veranderingen in cholesterol metabolisme en immunologische processen bijdragen aan het ontstaan van de ziekte van Alzheimer. De meest gangbare manieren om Alzheimer biomarkers tot nu toe vast te stellen is metingen van wiwitveranderingen in hersenvocht of  veranderingen gemeten met MRI of PET-scans. Er wordt gezocht naar makkelijker toegankelijk biomarkers, bijvoorbeeld in bloed, maar tot recent waren deze met wisselende betrouwbaarheid te meten.

**Voorstadia van de ziekte van Alzheimer**

Tot op heden is er nog geen therapie voor de ziekte van Alzheimer. Een van de mogelijke verklaringen hiervoor is dat de onderzoeken naar het opruimen van het amyloid eiwit uitgevoerd werden in patiënten met Alzheimer dementie. Een theorie is dat er in het dementie stadium al te veel onomkeerbare schade in de hersenen is. We weten inmiddels dat al 20 jaar voor een diagnose Alzheimer dementie de eerste neurodegeneratieve veranderingen beginnen. De wetenschap dat al jaren voor Alzheimerdementie de eerste veranderingen plaatsvinden en het ontbreken van een therapie maakt dat er steeds meer onderzoek gedaan wordt naar voorstadia van Alzheimer dementie.

Milde cognitieve stoornissen (mild cognitive impairment of MCI) refereert aan een pre-dementie stadium waarin er geheugenproblemen zijn, geobjectiveerd met een neuropsychologisch testonderzoek, maar er is nog geen interferentie in het dagelijks leven. Mensen met MCI hebben een verhoogd risico op dementie, ook is er vaker aanwezigheid van het amyloid eiwit ten opzichte van de normale populatie. Nog vroeger in het ziekteproces bekijkend, focust het onderzoeksveld zich steeds meer op het preklinische stadium van de ziekte Alzheimer. Met preklinische Alzheimer bedoelen we het stadium waarin er al Alzheimerveranderingen gaande zijn, bijvoorbeeld amyloid neerslag, maar het denkvermogen is nog niet objectief aangedaan, zoals gemeten met neuropsychologisch onderzoek (figuur 1). Sommige mensen met preklinische tekenen van Alzheimer hebben al wel geheugenklachten, ook wanneer dit nog niet vastgesteld kan worden met objectieve neuropsychologische testen.



Figuur 1. Hypothetisch model van dynamische biomarkers betrokken bij het ontstaan van de ziekte van Alzheimer (Sperling et al., 2011).

## Subjectieve geheugenklachten

Het concept Subjectieve Geheugenklachten, in het Engels *Subjective Cognitive Decline* (SCD) genoemd, beschrijft de subjectieve ervaring van achteruitgang in de cognitieve functies, terwijl er bij objectieve neuropsychologische testen geen afwijkingen zijn. Uit eerder onderzoek blijkt dat mensen met normale geheugenfuncties en aanwezigheid van geheugenklachten ten opzichte van mensen zonder geheugenklachten een verhoogd risico hebben op cognitieve achteruitgang, d.w.z. progressie naar milde cognitieve stoornissen of dementie. Daarom wordt ook wel gesuggereerd dat SCD een mogelijke eerste symptoom van preklinische Alzheimer is (figuur 2). Het is van belang om te benadrukken dat het grootste gedeelte van de mensen met geheugenklachten geen dementie ontwikkelt. Er zijn verschillende andere vaker voorkomende oorzaken van geheugenklachten, bijvoorbeeld somberheid, stress, verschillende leefstijlfactoren zoals slaap en alcohol, maar ook verschillende ziektes zoals een traag werkende schildklier of bepaalde medicijnen. Om de verschillende factoren die bijdragen aan subjectieve geheugenklachten te onderzoeken, evenals de relatie met preklinische Alzheimer, is het Subjective Cognitive Impairment Cohort (SCIENCe) opgezet in het Alzheimer Centrum Amsterdam.
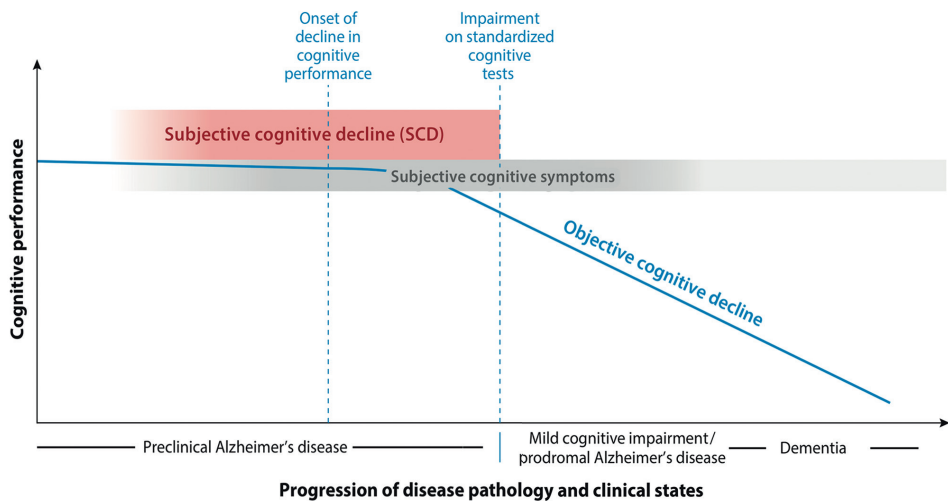


Figuur 2. Het concept subjectieve geheugenklachten (SCD) in de context van het beloop van de ziekte van Alzheimer.

**Doel van dit proefschrift**

In dit proefschrift onderzocht ik het concept Subjectieve Geheugenklachten (SCD) en biomarkers in voorstadia van Alzheimer dementie.

Dit proefschrift heeft drie hoofddoelen:

1. Het beschrijven van karakteristieken van mensen met SCD in het SCIENCe project.
2. Het onderzoeken van risicofactoren die bijdragen aan achteruitgang in geheugen in mensen met SCD.
3. Het evalueren van vroege biomarkers van de ziekte van Alzheimer en indicatoren van klinische achteruitgang in mensen met SCD.

**RESULTATEN**

**Deel 1: Karakteristieken van mensen met subjectieve geheugenklachten**

In **hoofdstuk 2** hebben we karakteristieken beschreven van mensen met subjectieve geheugenklachten uit het Subjective Cognitive Impairment Cohort (SCIENCe). Dit cohort hebben we opgezet in 2014 in het Alzheimer Centrum Amsterdam. In deze eerste cross-sectionele evaluatie van de eerste 150 onderzoeksdeelnemers met SCD vonden we een heterogene groep met verschillende karakteristieken. Ongeveer 25% van deze cognitief normale mensen had aanwijzingen voor preklinische Alzheimer, gedefinieerd als een verlaagd amyloid eiwit in hersenvocht of aanwezigheid van amyloid neerslag op een PET-scan. De aanwezigheid van preklinische Alzheimer nam toe met de leeftijd. Hoewel mensen met een bekende psychiatrische ziekte, zoals een depressie of een bipolaire stoornis, niet aan het onderzoek konden deelnemen, zagen we in ongeveer een derde van de deelnemers milde psychiatrische symptomen gemeten met vragenlijsten. Het grootste gedeelte van de deelnemers had geen specifieke kenmerken die geassocieerd zijn met geheugenklachten.

In dit artikel hebben we ook de SCD-plus criteria onderzocht. Deze criteria zijn door de internationale SCD-werkgroep voorgesteld als mogelijke indicatoren voor preklinische Alzheimer. We vonden dat SCD-plus criteria 'leeftijd boven de 60' en 'Apolipoprotein E (APOE) genotype' geassocieerd zijn met een verhoogd risico op preklinische Alzheimer. Momenteel is de inclusie en follow-up van deelnemers in de SCIENCe studie nog gaande en hopelijk zullen de vervolgresultaten meer inzicht geven.

**Deel II: risicofactoren van cognitieve achteruitgang in mensen met SCD**

In het tweede deel van dit proefschrift onderzochten we het risico op dementie en risicofactoren van achteruitgang in mensen met SCD, zowel op groepsniveau (**hoofdstuk 3**) als ook gepersonaliseerde risicofactoren (**hoofdstuk 4**).

Allereerst hebben we SCD in een breder perspectief bekeken. Ik onderzocht de incidentie (het aantal nieuwe gevallen van een ziekte per jaar) van dementie voor mensen met SCD en dit vergeleek met een controlegroep zonder geheugenklachten (**hoofdstuk 3**). Dit onderzoek werd uitgevoerd in een groot

internationaal samenwerkingsverband met meer dan vierduizend cognitieve normale deelnemers. Hiervoor combineerden we longitudinale gegevens van verschillende cohortstudies uit Europa, Australië en de Verenigde Staten. Op groepsniveau was de incidentie van dementie hoger in mensen met SCD vergeleken met mensen zonder geheugenklachten. Deze resultaten waren aanwezig zowel dementie ten gevolge van de ziekte van Alzheimer als ook andere vormen van dementie, bijvoorbeeld vasculaire dementie of frontotemporale dementie. Risicofactoren voor progressie naar dementie waren: een hogere leeftijd, lagere resultaten op geheugentesten (MMSE) en APOE e4-genotype dragerschap. Opvallend genoeg was het risico op dementie voor mensen met SCD hoger in een geheugenkliniek dan voor mensen met SCD onderzocht in een bevolkingsonderzoek.
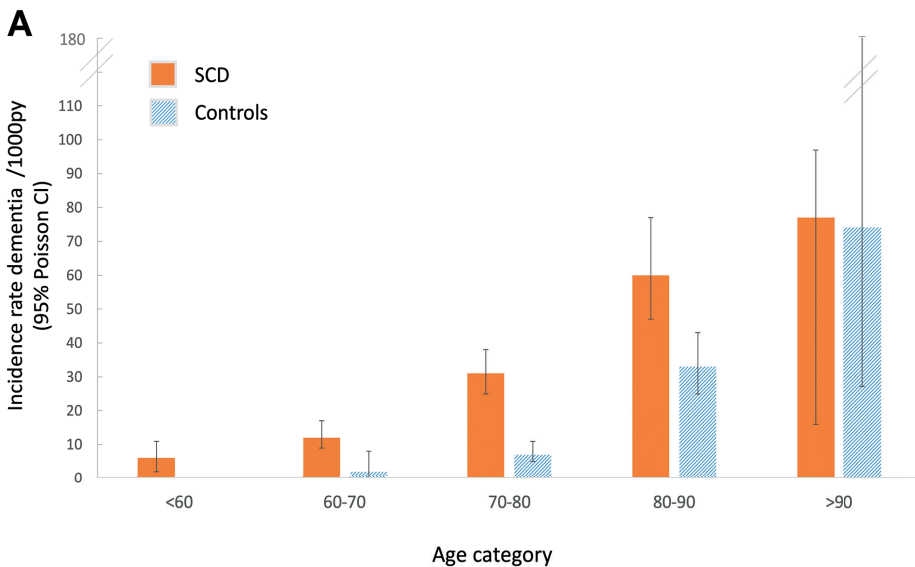


Figuur 3: Incidentie van dementie in cognitief normale mensen mét en zonder geheugenklachten per 1000 persoonsjaren.

Vervolgens onderzochten we in **hoofdstuk 4** het risico op MCI en dementie op individueel niveau in mensen SCD. We gebruikten hiervoor individuele patiëntgegevens zoals de eiwitten Abeta42 en tau in hersenvocht en MRI-metingen. We vonden dat normale Abeta42 en tau waarden in hersenvocht het risico op progressie naar dementie verlaagde, terwijl abnormale hersenvocht-waarden juist het risico verhoogde. MRI-waarden droegen niet bij aan het risico op progressie op individueel niveau.

**Deel III: Biomarkers van de ziekte van Alzheimer in pre-dementie stadia**

In het derde deel van dit proefschrift onderzocht ik de bijdrage van nieuwe biomarkers aan het risico op dementie ten gevolge van de ziekte van Alzheimer.

In **hoofdstuk 5** hebben we de relatie onderzocht tussen cholesterol transporter apolipoproteïne A1 (ApoA1) en het risico op klinische progressie naar MCI en dementie. In het lichaam is ApoA1 betrokken bij HDL-cholesterol transport. ApoA1 is van belang voor een goede lipidenbalans in het bloed. Er zijn aanwijzingen dat cholesterolveranderingen betrokken zijn bij het ontstaan van de ziekte van Alzheimer. Zo is het APOE gen, betrokken bij cholesterol metabolisme, een risicofactor voor Alzheimer dementie. We observeerden dat hogere ApoA1 waarden in hersenvocht en lagere ApoA1 waarden in bloedplasma geassocieerd waren met een verhoogd risico op MCI en dementie. Dit effect was sterker in mensen met een APOE e4 genotype.

We hebben ApoA1 en 2 andere cholesteroltransporter (apolipoproteïnen) verder geëvalueerd in **hoofdstuk 6**. We onderzochten in deze cross-sectionele studie het effect van cholesterol transporters ApoA1, apolipoproteïne E (ApoE) en apolipoproteïne J (clusterine) waarden in hersenvocht op de relatie tussen APOE genotype en hersenvochteiwitten Abeta42 en CSF. Hiervoor gebruikten we een statisch model, namelijk mediatie analyse met behulp van lineaire regressieanalyses. We vonden dat ApoE- en clusterinespiegels de relatie tussen APOE-genotype en tau beïnvloedden. Opmerkelijk was dat deze apolipoproteïnen de relatie tussen APOE-genotype en Alzheimereiwit amyloid niet beïnvloedden, ondanks de bekende sterke relatie tussen deze twee biomarkers. Dit suggereert dat ApoE en clusterin mogelijk bijdragen aan het ontstaan van de ziekte van Alzheimer via andere routes dan de bekende amyloid route, namelijk via beïnvloeding van het cholesterol metabolisme (figuur 4).

Als laatste beschreven we in **hoofdstuk 7** het amyloid eiwit gemeten in bloed met de nieuwe SIMOA-techniek. Tot voorkort waren resultaten van amyloid bepalingen in bloed tegenstrijdig, omdat er in het bloed veel verschillende verstorende factoren kunnen zijn. De nieuwe SIMOA techniek lijkt veelbelovend in het detecteren van veranderingen in bloed. We onderzochten in een statistisch model de toegevoegde waarde van afwijkend amyloid in bloed op het identificeren van preklinische Alzheimer (afwijkend amyloid in hersenvocht en op PET-scans). Amyloid (abeta42 en abeta40) gemeten in bloed met de SIMOA methode bleek in dit statische model afwijkende amyloid waardes in hersenvocht en plasma te kunnen voorspellen. Dit is een van de eerste keren dat dit zo beschreven wordt. Ook vonden we op groepsniveau dat amyloid veranderingen in bloed (de Abeta42/Abeta40 ratio) al in cognitief gezonde mensen met geheugenklachten gerelateerd is aan een verhoogd risico op klinische achteruitgang naar MCI of dementie. De resultaten van deze studie benadrukken de potentie en relevantie van in bloed gemeten Abeta42 en Abeta40 waarden om preklinische Alzheimer in SCD te identificeren.
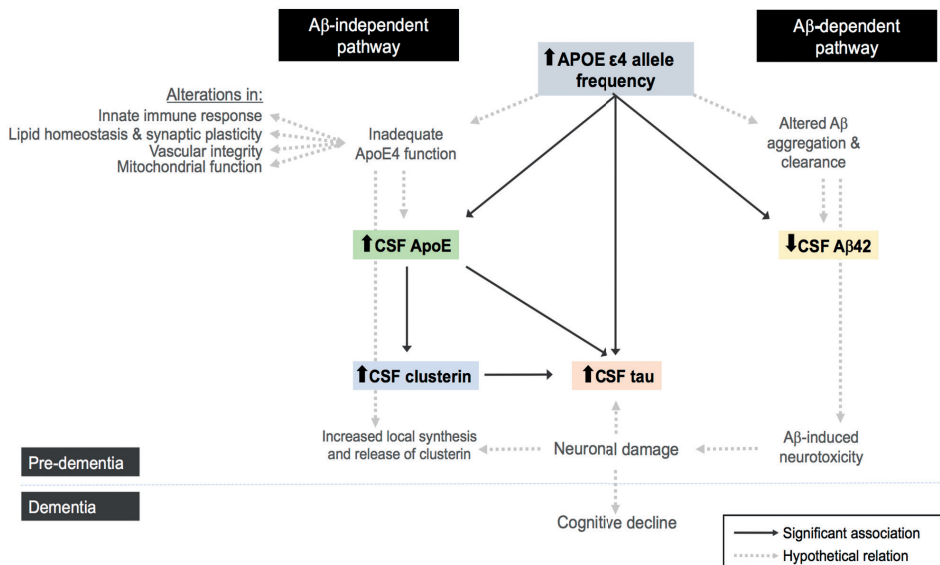
Figuur 4. Het effect van cholesteroletransporters ApoA1, ApoE en ApoJ op de relatie tussen risicofactor APOE e4 genotype en Alzheimereiwitten in hersenvocht (Slot et al., Neurobiology of Aging, 2019).

## CONCLUSIES

Dit proefschrift onderschrijft het belang van het concept van SCD vanuit zowel een onderzoeks- als een klinisch perspectief. Onze bevindingen geven aan dat SCD niet alleen een goedaardige aandoening is. Hoewel de meerderheid van de mensen met geheugenklachten zonder objectieve geheugenstoornissen geen neurodegeneratieve ziekte heeft, observeerden we in een van de vier SCD-deelnemers aan onze studies preklinische Alzheimer. Ook zagen we dat het risico op progressie naar dementie hoger is in SCD dan bij personen zonder cognitieve klachten. Specifieke kennis van onderliggende oorzaken van cognitieve klachten bij SCD, zoals preklinische Alzheimer, of bijvoorbeeld milde psychiatrische symptomen, kan helpen bij het bepalen van vervolg stappen voor het individu met SCD. Hopelijk komt met verder onderzoek naar SCD en preklinische Alzheimer een vroeg medicijn voor de ziekte dichterbij. Voor mensen met SCD zonder aanwijzingen voor preklinische Alzheimer zouden interventies zoals psycho-educatie of leefstijlveranderingen een verbetering kunnen bewerkstellen. Verder onderzoek hiernaar is nodig.

In onze biomarker studies observeerden we dat veranderingen in cholesteroltransporters apolipoproteïne A, E en J in hersenvocht en plasma in niet-demente mensen geassocieerd zijn met een verhoogd risico op Alzheimer dementie. Ook observeerden we dat veranderingen van de eiwitten Abeta40 en Abeta42 in bloedplasma gemeten met SIMOA een veelbelovende pre-screening instrument kan zijn om in gezonde mensen met geheugenklachten toekomstige

achteruitgang te voorspellen.

In de nabije toekomst zullen hopelijk gepersonaliseerde risicoscores en vroege Alzheimer screeningtools de huisarts in de praktijk helpen bij het bepalen of een persoon met geheugenklachten moet worden doorverwezen naar een gespecialiseerde geheugenkliniek. Uiteindelijk is het doel om vast te stellen welke cognitief normale mensen met een hoog risico op Alzheimer in aanmerking komen voor behandeling. Wanneer nauwkeurige risicoschattingen voor het individu kunnen worden gemaakt, kunnen gepersonaliseerde preventieve interventies worden ingezet.

## LIST OF AFFILATIONS

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Verfaillie SCJ, Timmers T, **Slot RER**, van der Weijden CWJ, Wesselman LMP, Prins ND, Sikkes SAM, Yaqub M, Dols A, Lammertsma AA, Scheltens P, Ossenkoppele R, van Berckel BNM, van der Flier WM.
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**Longitudinal cerebrospinal fluid biomarker trajectories along the Alzheimer's disease continuum in the BIOMARKAPD study.**
Lleó A, Alcolea D, Martínez-Lage P, Scheltens P, Parnetti L, Poirier J, Simonsen AH, Verbeek MM, Rosa-Neto P, **Slot RER**, Tainta M, Izaguirre A, Reijs BLR, Farotti L, Tsolaki M, Vandenberge R, Freund-Levi Y, Verhey FRJ, Clarimón J, Fortea J, Frolich L, Santana I, Molinuevo JL, Lehmann S, Visser PJ, Teunissen CE, Zetterberg H, Blennow K.
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**Amyloid PET and cognitive decline in cognitively normal individuals: the SCIENCe project.**
Timmers T, Ossenkoppele R, Verfaillie SCJ, van der Weijden CWJ, **Slot RER**, Wesselman LMP, Windhorst AD, Wolters EE, Yaqub M, Prins ND, Lammertsma AA, Scheltens P, van der Flier WM, van Berckel BNM.
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**High amyloid burden is associated with fewer specific words during spontaneous speech in individuals with subjective cognitive decline.**
Verfaillie SCJ, Witteman J, **Slot RER**, Pruis IJ, Vermaat LEW, Prins ND, Schiller NO, van de Wiel M, Scheltens P, van Berckel BNM, van der Flier WM, Sikkes SAM.
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**Dietary Patterns Are Related to Clinical Characteristics in Memory Clinic Patients with Subjective Cognitive Decline: The SCIENCe Project.**
Wesselman LMP, Doorduijn AS, de Leeuw FA, Verfaillie SCJ, van Leeuwenstijn-Koopman M, **Slot RER**, Kester MI, Prins ND, van de Rest O, de van der Schueren MAE, Scheltens P, Sikkes SAM, van der Flier WM.
*Nutrients. 2019 May 11;11(5). pii: E1057. doi: 10.3390/nu11051057.*

**ATN-classification and clinical progression in subjective cognitive decline: the SCIENCe project.**
Jarith L. Ebenau; Tessa Timmers; Linda M.P. Wesselman; Inge M.W. Verberk,; Sander C.J. Verfaillie; **Rosalinde E.R. Slot**; Argonde C. van Harten; Charlotte E. Teunissen,; Frederik Barkhof; Karlijn A. van den Bosch; Mardou van Leeuwenstijn; Jori Tomassen; Anouk den Braber; Pieter Jelle Visser; Niels D. Prins; Sietske A.M. Sikkes; Philip Scheltens; Bart N.M. van Berckel; Wiesje M. van der Flier. *Submitted*.

**Persisterende subjectieve geheugenklachten: neurologie of psychiatrie?**
Melissa van der Sluijs; Hans Jeuring; Caroline Sonnenberg; Nicole Korten; **Rosalinde Slot**; Sander Verfaillie; Jozefien M. Overbeek; Wiesje M. van der Flier; Annemieke Dols. *Submitted*

**LIST OF THESES 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)
11. A. Bastos Leite  Pathological ageing of the brain (16.11.2007)
12. E. van Straaten  Vascular dementia (11.01.2008)
13. R. Vogels  Cognitive impairment in heart failure (11.04.2008)
14. J. Damoiseaux  The brain at rest (20.05.2008)
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)
24. N. Verwey  Biochemical markers in dementia: from mice to men – a translational approach (15.04.2010)
25. M. Kester  Biomarkers for Alzheimer's pathology – monitoring, predicting and understanding the disease (14.01.2011)
26. J. Sluimer  Longitudinal changes in the brain (28.04.2011)
27. S. Mulder  Amyloid associated proteins in Alzheimer's disease (07.10.2011)
28. S. Sikkes  Measuring IADL in dementia (14.10.2011)
29. A. Schuitemaker  Inflammation in Alzheimer's disease – in vivo quantification (27.01.2012)
30. K. Joling  Depression and anxiety in family caregivers of persons with dementia (02.04.2012)
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)
48. C. Möller  Imaging patterns of tissue destruction – towards a better discrimination of types of dementia (01.05.2015)
49. M. del Campo Milán  Novel biochemical signatures of early stages of Alzheimer's disease (19.06.2015)
50. M. Benedictus  A vascular view on cognitive decline and dementia: relevance of cerebrovascular MRI markers in a memory clinic (20.01.2016)
51. M. Zwan  Visualizing Alzheimer's disease pathology – implementation of amyloid PET in clinical practice (03.03.2016)
52. E. Louwersheimer  Alzheimer's disease – from phenotype to genotype (21.06.2016)
53. W. Krudop  The frontal lobe syndrome – a neuropsychiatric challenge (23.09.2016)
54. E. Vijverberg  The neuropsychiatry of behavioural variant frontotemporal dementia and primary psychiatric disorders – similarities and dissimilarities (22.09.2017)
55. F. Gossink  Late onset behavioral changes – differentiating between bvFTD and psychiatric disorders in clinical practice (20.04.2018)
56. M. Engels  Neurophysiology of dementia – the resting-state of the art (18.05.2018)
57. S. Verfaillie  Neuroimaging in subjective cognitive decline – incipient Alzheimer's disease unmasked (12.09.2018)
58. M. ten Kate  Neuroimaging in predementia Alzheimer's disease (13.09.2018)
59. H. Rhodius-Meester  Optimizing use of diagnostic tests in memory clinics – the next step (24.09.2018)
60. E. Willemse  Optimizing biomarkers in cerebrospinal fluid – how laboratory reproducibility improves the diagnosis of Alzheimer's disease (18.10.2018)
61. E. Konijnenberg  Early amyloid pathology – identical twins, two of a kind? (25.06.2019)
62. A. Leeuwis  Connecting heart and brain – vascular determinants of cognitive impairment and depressive symptoms (02.07.2019)
63. J. den Haan  Imaging the retina in Alzheimer's disease (12.09.2019)

64. A. van Loenhoud  Cognitive reserve in Alzheimer's disease – a perspective on the flourishing and withering brain (18.09.2019)
65. R. Jutten  Capturing changes in cognition – refining the measurement of clinical progression in Alzheimer's disease (20.09.2019)
66. N. Legdeur  Determinants of cognitive impairment in the oldest-old (08-10-2019)
67. R. Slot  Subjective Cognitive Decline – predictive value of biomarkers in the context of preclinical Alzheimer's disease (14-11-2019)

## PORTFOLIO

Alzheimer center & department of Neurology, Amsterdam UMC, Vrije Universiteit Amsterdam

PhD period:      October 2013 – June 2017
Promotors:       Prof.Dr. W.M. van der Flier
                 Prof.Dr. Ph. Scheltens
Copromotor:      Prof.Dr.Ir. C.E. Teunissen

| Courses | Year | ECTS |
|---|---|---|
| Basic Course for Clinical Investigators (BROK) | 2014 | 1.5 |
| Participation Friday PhD program Alzheimer Center Amsterdam | 2013-2017 | 8.0 |
| **International Conferences** | | |
| Alzheimer's Association Internation Conference (AAIC) Copenhagen: oral presentation | 2014 | 1.5 |
| AAIC Washington: oral and poster presentation | 2015 | 2.5 |
| AAIC Toronto: oral and poster presentation | 2016 | 2.5 |
| AAIC London: oral presentation | 2017 | 1.5 |
| **National conferences** | | |
| Dementia update Amsterdam | 2014-2016 | 0.9 |
| NCA Annual meeting Amsterdam, poster presentation | 2014-2016 | 1.9 |
| Biannual NUBIN meeting on biomarkers in neurodegenerative diseases Amsterdam: oral presentation | 2014 | 1.5 |
| TN2 conference Amsterdam | 2015 | 0.3 |
| Mix&Match meeting Alzheimer Nederland | 2014-2016 | 0.9 |
| Nederlandse Vereniging voor Neurologie Wetenschapsdagen: oral presentation | 2017 | 1.5 |
| **Other** | | |
| Clinical work as resident neurology Department of Neurology and Alzheimer center Amsterdam | 2013-2017 | 6.0 |
| **Teaching** | | |
| Tutor bachelor students medicine (3rd year): one semester | 2015 | 2.0 |
| Basic training Neurology master students medicine | 2013-2016 | 4.0 |
| Lecture in the course neurodegeneration for bachelor students medicine (3rd year) | 2016 | 1.0 |

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**ABOUT THE AUTHOR**

Rosalinde Slot was born on the 21st of October 1985 in Den Haag. She grew up in Deventer where she obtained her Gymnasium diploma at the Geert Groote College in 2004. In 2004 she studied Spanish in Salamanca, Spain, followed by practising her Spanish whilst travelling through South America. In 2005 she started studying Pharmaceutical Sciences at the University of Utrecht. From 2006 to 2012 she studied Medicine at the VU University Medical Center, Amsterdam. During her studies she developed an interest in Neurology, especially cognitive Neurology, which resulted in a scientific internship at the Alzheimer Center Amsterdam, studying disease severity in frontotemporal dementia under supervision of Dr. Yolande Pijnenburg. In February 2013 she started working as resident Neurology at the Slotervaart hospital, Amsterdam. At the end of 2013 she started her PhD project on Subjective Cognitive Decline under supervision of Prof. Dr. Wiesje van der Flier, Prof. Dr. Philip Scheltens and Prof. Dr. Ir. Charlotte Teunissen at the Alzheimer Center Amsterdam, VU University Medical Center. During her PhD project Rosalinde set up a longitudinal cohort (SCIENCe project), structurally evaluating individuals with Subjective Cognitive Decline, and investigating CSF and plasma biomarkers of (preclinical) Alzheimer's Disease, which resulted in this thesis. In July 2017 she started her specialty registrar training in Neurology in the VU University Medical Center, now Amsterdam UMC, under supervision of Prof. Dr. H.W. Berendse, and in the Onze Lieve Vrouwe Gasthuis Amsterdam, under supervision of Prof. Dr. H.C. Weinstein. Rosalinde lives in Amsterdam with her husband Karl Breukink.