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## The Journal of Prevention of Alzheimer's Disease

journal homepage: [www.elsevier.com/locate/tjpad](http://www.elsevier.com/locate/tjpad)

## Original Article

# Plasma A $\beta$ 42/A $\beta$ 40 determined by mass spectrometry is associated with longitudinal changes in amyloid accumulation, brain atrophy, and conversion to mild cognitive impairment due to Alzheimer's disease in individuals with subjective cognitive decline: 5-year follow-up of the FACEHBI cohort

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## ARTICLE INFO

## Keywords:

Alzheimer's disease  
A $\beta$ 42/A $\beta$ 40  
Blood biomarkers

## ABSTRACT

**Background:** The accurate identification of individuals at risk of Alzheimer's disease (AD) through blood-based biomarkers remains challenging.

**Objectives:** To evaluate the association between plasma amyloid-beta (A $\beta$ )42/A $\beta$ 40 ratio and longitudinal amyloid deposition, clinical progression, brain atrophy and cognitive decline.

**Abbreviations:** A $\beta$ , Amyloid- $\beta$ ; AD, Alzheimer's disease; APOE, Apolipoprotein E; AUC, Area Under the ROC Curve; CH, Cognitively healthy; CI, Confidence interval; CL, Centiloid; CSF, Cerebrospinal fluid; FACEHBI, Fundació ACE Healthy Brain Initiative; FBB, <sup>18</sup>F-Florbetaben; GFAP, Glial fibrillary acidic protein; HR, Hazard ratio; IP-MS, Immunoprecipitation- mass spectrometry method; IQR, Interquartile range; LMEMs, Linear mixed-effects models; MCI, Mild cognitive impairment; MMSE, Mini-Mental State Examination; MRI, Magnetic resonance imaging; MS, Mass spectrometry; NBACE, Neuropsychological battery of Fundació ACE; NfL, Neurofilament light; p-Tau, Phosphorylated tau; PET, Positron emission tomography; ROC, Receiver Operating Characteristic; SCD, Subjective cognitive decline; S-FNAME, Spanish version of the Face-Name Associative Memory Exam; SUVR, Standard uptake value ratio.

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<sup>2</sup> Data used in the preparation of this article were obtained from the AMYPAD project. As such, the investigators within AMYPAD contributed to the design and implementation of AMYPAD and/or provided data but did not participate in the analysis or writing of this report. A complete listing of AMYPAD contributors can be found at: <https://amypad.eu/partners/>

<https://doi.org/10.1016/j.tjpad.2025.100465>

Received 12 June 2025; Received in revised form 5 December 2025; Accepted 20 December 2025

Available online 8 January 2026

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Mass spectrometry  
Subjective cognitive decline

**Design, setting and participants:** This study extends the Fundació ACE Healthy Brain Initiative (FACEHBI) study (Barcelona, Spain), comprising 200 individuals with subjective cognitive decline (SCD) followed over five years. **Measurements:** A $\beta$ 42/A $\beta$ 40 ratio was quantified using ABtest-MS, an antibody-free mass-spectrometry (MS) method. Survival analyses compared conversion risks to amyloid-PET positivity and mild cognitive impairment (MCI), in participants classified as low or high A $\beta$ 42/A $\beta$ 40, based on a cutoff of  $\leq 0.241$ . Linear mixed-effect models evaluated associations of this biomarker with longitudinal changes in amyloid deposition, brain volume, and cognition.

**Results:** Low baseline A $\beta$ 42/A $\beta$ 40 was significantly associated with increased amyloid accumulation ( $\beta = 0.257$ , 95% confidence interval (CI) 0.177–0.336,  $P < 0.001$ ), and with higher risk of conversion to A $\beta$ -PET positivity (Hazard ratio (HR) = 2.84, 95% CI 1.14–7.04,  $P = 0.025$ ) and to MCI due to AD (HR = 3.25, 95% CI 1.17–9.01,  $P = 0.024$ ). It was also linked to decreased hippocampal ( $\beta = -1.183$ , 95% CI -2.154 to -0.211,  $P = 0.017$ ) and cortical ( $\beta = -75.921$ , 95% CI -151.728 to -0.113,  $P = 0.050$ ) volumes, and increased ventricular volume ( $\beta = 35.175$ , 95% CI 18.559–51.790,  $P < 0.001$ ). Moreover, lower baseline levels of A $\beta$ 42/A $\beta$ 40 were weakly associated with greater worsening in Mini-Mental State Examination and complex associative memory.

**Conclusions:** Our findings suggest that the plasma A $\beta$ 42/A $\beta$ 40 ratio is associated with future amyloid accumulation, brain atrophy, and conversion to prodromal AD in individuals with SCD. This biomarker may help characterize individuals with a higher likelihood of progression and could support earlier and more personalized strategies.

## 1. Introduction

As one of the most pressing public health challenges of our time, Alzheimer's disease (AD) is a complex neurodegenerative disorder defined by the accumulation of extracellular amyloid-beta (A $\beta$ ) plaques and intracellular hyperphosphorylated tau tangles, which culminate in cognitive impairment and dementia [1]. AD causes 60–70% of dementia cases, affecting 57 million people worldwide [2]. This number is expected to triple by 2050 due to the aging population [3], highlighting the critical need for effective interventions.

AD is characterized by an extended asymptomatic phase, with amyloid pathology being recognized as the earliest pathophysiological change [4]. Indeed, only after amyloid accumulation becomes abnormal, other biomarkers such as those related to tau pathology, neuroinflammation, synaptic dysfunction, and neurodegeneration become altered [5].

Cumulative evidence suggests that current disease-modifying therapies could be more effective in individuals at less advanced stages of the disease [6,7]. For this reason, ongoing clinical trials with A $\beta$ -targeting monoclonal antibodies are being conducted in cognitively healthy (CH) individuals who show evidence of early AD pathology [8,9].

The advances in treatment options have forced rapid progression in the field of diagnostics, as the correct identification of individuals who could benefit most from these therapies is of utmost importance. The diagnosis of AD has been primarily based on cerebrospinal fluid (CSF) biomarkers, such as A $\beta$ 42 [10], total tau (t-tau) and phosphorylated tau (p-tau) [11], or neuroimaging techniques like positron emission tomography (PET), to assess amyloid pathology [12]. While these methods have proven diagnostic value, they are invasive, costly, and not suitable for routine clinical practice, longitudinal studies, and widespread screening.

In recent years, blood-based biomarkers have gained attention as non-invasive, accessible, and cost-effective alternatives, more appropriate for large-scale screening of at-risk individuals. Among these, the plasma A $\beta$ 42/A $\beta$ 40 ratio has shown a strong association with cortical amyloid burden, as measured by PET imaging or CSF analysis [13–15], indicating that this biomarker may offer a viable alternative to invasive methods. Furthermore, as a marker of amyloidosis, it has the potential to identify individuals at risk for AD years before clinical symptoms appear, making it a valuable tool for early detection.

However, reliable quantification of these peptides in plasma, especially A $\beta$ 42, has been an ongoing challenge [16], due to its low concentration and propensity for aggregation with other highly abundant compounds in this matrix. In addition, the significant overlap in plasma A $\beta$ 42/A $\beta$ 40 values between cortical amyloid-positive and

amyloid-negative groups [17] introduces an additional challenge.

Recent advancements in mass spectrometry (MS) and immunoassays have significantly improved the sensitivity, precision, and reliability of plasma A $\beta$  measurements [18,19]. Plasma MS-based assays offer advantages over immunoassays in terms of accuracy, as evidenced in head-to-head studies [20].

In recent years, increasing attention has been paid to the potential of blood biomarkers to predict longitudinal changes in cumulative amyloidosis, brain atrophy, and cognitive function [21–25]. Nevertheless, despite these advancements, data from studies utilizing highly reliable techniques remain limited, underscoring the need for more comprehensive research.

This study provides a 5-year extension of previously published data (see Pascual-Lucas et al., 2023 [15], with results from the baseline visit and the 2-year follow-up visit) from the Fundació ACE Healthy Brain Initiative (FACEHBI) cohort, which includes 200 CH individuals with subjective cognitive decline (SCD). This population, characterized by self-reported cognitive decline without objective deficits on standardized cognitive tests [26], has gained attention due to its association with an increased risk of developing mild cognitive impairment (MCI) and eventually progressing to dementia [27]. In this longitudinal study, we explored whether the plasma A $\beta$ 42/A $\beta$ 40 ratio, measured at baseline using an antibody-free MS technique (ABtest-MS; Araclon Biotech), is associated with: a) amyloid accumulation, as assessed by A $\beta$ -PET; b) clinical conversion from SCD to MCI; c) changes in brain volumes, measured by magnetic resonance imaging (MRI); and d) cognitive decline, assessed through the Mini-Mental State Examination (MMSE) and several cognitive composites derived from an extensive battery of neuropsychological tests.

## 2. Methods

### 2.1. Participants

FACEHBI is a long-term, single-center, prospective observational study conducted at Ace Alzheimer Center Barcelona (Spain) aimed at characterizing a population of subjects with SCD. One of the main objectives of the study is to determine which clinical, genetic, neuropsychological, biochemical, and neuroimaging variables are the best predictors of cognitive and functional impairment over time in individuals with SCD [28].

A total of 200 individuals with SCD diagnosis over the age of 49 were initially enrolled in this study. Data from baseline to 5-year follow-up visit (V5) are presented in this manuscript. Blood collection and complete neurological and neuropsychological examinations, including clinical diagnosis (SCD or MCI), were performed at each annual visit.

Further details regarding the criteria used for SCD and MCI diagnosis are provided in the Supplementary Material (section “Diagnosis criteria for SCD and MCI”).

An  $^{18}\text{F}$ -florbetaben (FBB)-PET, used to identify cortical amyloid load, and a brain MRI scan, used to assess brain atrophy and vascular pathology, were included at the baseline visit, as well as at the 2-year (V2) and 5-year (V5) follow-ups. A more detailed description of the procedures associated with this study is included in the Supplementary Information of Pascual-Lucas et al. 2023 [15]. Further information about FACEHBI study design and inclusion/exclusion criteria can be found elsewhere [28].

## 2.2. Plasma A $\beta$ analyses

Plasma samples from baseline visit (V0) were collected between December 2014 and March 2016. Plasma A $\beta$ 40 and A $\beta$ 42 were quantified using ABtest-MS in July 2021. This method is an antibody-free high-performance liquid chromatography-differential mobility spectrometry-triple quadrupole mass-spectrometry (HPLC-DMS-MS/MS) method developed by Araclon Biotech (Zaragoza, Spain). Briefly, analytes were extracted directly from plasma since no immunoprecipitation (IP) procedure was followed. Intact A $\beta$ 40 and A $\beta$ 42 species were measured as no enzymatic digestion was required. Deuterated internal standards ( $^2\text{H}$ -A $\beta$ 40 and  $^2\text{H}$ -A $\beta$ 42, Bachem AG, Bubendorf, Switzerland) were spiked in all samples, and response ratios corresponding to the endogenous species in study samples ( $^{14}\text{N}$ -A $\beta$ 40/ $^2\text{H}$ -A $\beta$ 40 and  $^{14}\text{N}$ -A $\beta$ 42/ $^2\text{H}$ -A $\beta$ 42) were interpolated in the calibration curves. Further details about the analytical procedure and instrumental acquisition parameters, as well as results about sensitivity, parallelism, accuracy and precision are described in the literature [15].

To identify individuals with amyloid deposition, a cutoff of 0.241 was previously established for plasma A $\beta$ 42/A $\beta$ 40 based on the analysis of the baseline visit of the FACEHBI cohort [15]. This cutoff was calculated at the maximum Youden index for amyloid deposition after Receiver Operating Characteristic (ROC) analysis (Area Under the ROC Curve (AUC) 0.87, 95 % confidence interval (CI) 0.80–0.93; sensitivity 86.1 %, specificity 80.5 %, positive predictive value 49.2 % and negative predictive value 96.4 %). Individuals with baseline plasma A $\beta$ 42/A $\beta$ 40 values  $\leq$  0.241 ( $n = 65$ ) were classified as low A $\beta$ 42/A $\beta$ 40, whereas those with A $\beta$ 42/A $\beta$ 40 values  $>$  0.241 ( $n = 135$ ) were identified as high A $\beta$ 42/A $\beta$ 40.

Alternative dual-cutoff strategies, which introduce an ‘indeterminate’ zone, were not applied in this study. Such approaches can help reduce misclassification around the threshold and may provide additional value in a diagnostic context. However, the aim of the present analysis was not to establish a diagnostic classification system, but rather to examine longitudinal trajectories of individuals stratified according to a single, predefined cutoff of plasma A $\beta$ 42/A $\beta$ 40 positivity.

## 2.3. $^{18}\text{F}$ -Florbetaben positron emission tomography (FBB-PET)

Detailed information about FBB-PET acquisition has been previously described [15]. In brief, a single dose of 300 Mbq of the FBB radiotracer (NeuraCeq) was administered. The standard uptake value ratio (SUVR) was calculated using the mean values from the cortical regions segmented on MRI. The cerebellum was used as the reference region for normalization. Centiloid (CL) values were calculated according to published procedures [29], considering the early amyloid deposition value of 13.5 CL as the threshold for positivity [30]. The intervals between the scan performed at baseline and those performed at the 2-year and 5-year follow-up visits were 25.3 [24.4–26.4] and 63.9 [62.3–65.7] months, (median [interquartile range, IQR]), respectively.

## 2.4. Brain MRI

A detailed explanation of the MRI acquisition process has been previously published [15]. Three different MRI parameters were measured: 1) hippocampal volume, defined as the mean of the left and right hippocampal volumes; 2) cortical volume and 3) total ventricular volume, calculated as the sum of the volumes of the left lateral ventricle, left inferior lateral ventricle, right lateral ventricle, right inferior lateral ventricle, third ventricle, and fourth ventricle. Additionally, the estimated total intracranial volume was also calculated, which was used to normalize the former three parameters (hippocampal, cortical and ventricular volumes). The median [IQR] time between baseline and follow-up scans was 24.8 [24.4–25.3] and 61.9 [60.6–64.1] months, for V2 and V5 respectively.

## 2.5. Clinical outcome measures

Participants from the FACEHBI cohort were administered an extensive neuropsychological assessment which included the MMSE [31,32], the Neuropsychological battery of Fundació ACE (NBACE) [33,34], and additional tests such as the Spanish version of the Face-Name Associative Memory Exam (S-FNAME) [35]. The FNAME is an associative memory test created to detect memory deficits in individuals with pre-clinical AD [36]. Twelve cognitive composites were calculated at baseline and follow-up V2 and V5 using data from MMSE, NBACE and S-FNAME scores (more detailed information can be found in **Supplementary Materials - Cognitive Composites section**). For the present study, data from MMSE and ten composites (Memory - Verbal, Memory - S-FNAME - Names, Memory - S-FNAME - Occupations, Memory - Visual, Language, Processing speed, Executive functions, Visuospatial/Visuospatial, Praxis and Attention) were used for the analysis. Additionally, at follow-up V2 and V5 a clinical diagnosis was assigned to each participant according to the information gathered by the neurologist regarding the individual’s cognition and functionality and performance on NBACE [33,34]. Of note, S-FNAME scores were not used for diagnosis assessment.

## 2.6. Statistical analyses

Statistical analyses and graphical representations of the data were conducted using GraphPad Prism v5.03 (GraphPad Software, San Diego, CA, USA) and SPSS v18 (IBM, Armonk, NY, USA), with the exception of linear mixed-effects models (LMEs). For these models, R software (version 4.4.2) was utilized, specifically the lme() function from the nlme package. A two-tailed  $P$ -value of  $<0.05$  was considered statistically significant.

To compare different groups, the Chi-square test or Fisher’s exact test (when appropriate) was used for categorical variables, and the Mann-Whitney U test was applied for continuous variables. The Bonferroni correction was applied to adjust the significance level for multiple comparisons. The association between two continuous variables was assessed using Spearman’s rank correlation coefficient. Changes in the variables throughout the study were represented as the difference between the data at the 5-year follow-up (V5) and the data at baseline (V0).

Kaplan-Meier survival analysis and Log-rank tests were performed to assess the association between baseline A $\beta$ 42/A $\beta$ 40 ratio in plasma and conversion to A $\beta$ -PET positivity during the follow-up. Additionally, adjusted and unadjusted Cox regression models were fitted, together with hazard ratios (HR) with 95 % CI. Age and APOE (number of  $\epsilon 4$  alleles) were included as covariates in the adjusted models. The “time-to-event” variable was defined as the time from baseline to conversion to A $\beta$ -PET+ or the time from baseline to last assessment in the case of those

who remained as A $\beta$ -PET $^-$ . For these analyses, only A $\beta$ -PET $^-$  subjects at baseline were included ( $n = 164$ ), as they were the population at risk of conversion. The same procedure was followed to evaluate the association between baseline plasma A $\beta$ 42/A $\beta$ 40 values and either conversion to all-cause MCI or conversion to MCI and A $\beta$ -PET $^+$  (hereafter referred to as "MCI due to AD" in the text). In the last case, conversion time was defined as the later of the two events: either the date of MCI conversion or the date of A $\beta$ -PET $^+$  conversion. The 200 SCD participants were initially included in both analyses as they were all classified as SCD at baseline and thus, at risk of progressing to MCI during the follow-up.

LMEMs were used to assess the association between baseline plasma A $\beta$ 42/A $\beta$ 40 values and the trajectories (longitudinal changes) of A $\beta$ -PET, volumetric MRI measurements and cognitive decline over time. The models included participant-specific random intercepts and time-specific random slopes, allowing for individual variation in the rate of change throughout the follow-up period. Age, APOE status (number of  $\epsilon$ 4 alleles), plasma A $\beta$ 42/A $\beta$ 40, time and the interaction term "A $\beta$ 42/A $\beta$ 40  $\times$  time" were included as fixed effects.  $\beta$  coefficient, 95 % CI and the  $P$ -value of this interaction term are reported, as it reflects the effect of plasma A $\beta$ 42/A $\beta$ 40 changes over time. A $\beta$ 42/A $\beta$ 40 data were included in a dichotomized format (high and low A $\beta$ 42/A $\beta$ 40, using the previously established cutoff of 0.241). Some analysis were also performed considering A $\beta$ 42/A $\beta$ 40 as a continuous variable. An unstructured covariance matrix was used to model the correlation structure.

### 3. Results

#### 3.1. Characteristics of the study population

At baseline, the 200 individuals enrolled in the study had a median [IQR] age of 67.0 [60.0–70.0] years, 63 % ( $n = 126$ ) were women, and 26 % ( $n = 52$ ) were APOE  $\epsilon$ 4 carriers. The prevalence of A $\beta$ -PET positivity (cutoff > 13.5 CL) was 18 % ( $n = 36$ ) and the median [IQR] MMSE score was 29.5 [29.0–30.0]. The study sample consisted entirely of participants of Caucasian ethnicity.

During the five years of follow-up, 30.5 % ( $n = 61$ ) of participants withdrew from the study: 3.5 % ( $n = 7$ ) at the 1-year follow-up, 3 % ( $n =$

6) at the 2-year follow-up, 14 % ( $n = 28$ ) at the 3-year follow-up, 3 % ( $n = 6$ ) at the 4-year follow-up and 7 % ( $n = 14$ ) at the 5-year follow-up. The reasons for participant withdrawal are provided in **Supplementary Table 1**. For the 193 participants with at least one follow-up visit, the mean (SD) duration of monitoring was 4.5 (1.3) years, with an average number of follow-up visits of 4.3 (1.2).

**Table 1** provides baseline characteristics of the study participants for the whole population and for the high and low A $\beta$ 42/A $\beta$ 40 groups. The participants with low A $\beta$ 42/A $\beta$ 40 at baseline were older (median [IQR]: 69.0 [65.0–73.0] vs 64.0 [60.0–69.0] years,  $P = 0.001$ ), had a lower proportion of females (47.7 % vs 70.4 %,  $P = 0.002$ ) and a higher frequency of APOE  $\epsilon$ 4 carriers (41.5 % vs 18.5 %,  $P = 0.002$ ). They also had higher A $\beta$ -PET CL values (11.39 [–2.17–35.37] vs –3.70 [–7.92–1.69] CL,  $P < 0.001$ ) and higher ventricular volume (28,119.5 [22,732.5–34,933.1] vs 24,319.2 [18,727.2–32,028.2] mm $^3$ ,  $P = 0.049$ ). Plasma levels of A $\beta$ 40, A $\beta$ 42 and A $\beta$ 42/A $\beta$ 40 ratio were also lower in the low A $\beta$ 42/A $\beta$ 40 group ( $P = 0.004$ ,  $P < 0.001$  and  $P < 0.001$  respectively).

The results of MMSE (**Table 1**) and the cognitive composites (**Supplementary Table 2**) performed at baseline were compared between the high and low A $\beta$ 42/A $\beta$ 40 groups. While MMSE scores were not different between the two groups, several cognitive composites (Memory - Verbal, Memory - S-FNAME Names, Memory - Visual, Praxis and Attention) showed statistically significant differences between high and low A $\beta$ 42/A $\beta$ 40 groups at baseline, reflecting worse cognitive performance in those individuals with lower A $\beta$ 42/A $\beta$ 40 values.

#### 3.2. Association between baseline A $\beta$ 42/A $\beta$ 40 and conversion to A $\beta$ -PET $^+$ at 5-year follow-up

In this study, 36 individuals (18 %) were enrolled as A $\beta$ -PET $^+$ , and 24 additional participants converted over the follow-up (6 of them at the 2-year visit and 18 at the 5-year visit) (**Table 2**). At baseline, plasma A $\beta$ 42/A $\beta$ 40 values were significantly lower ( $P = 0.028$ ) in the 24 subjects who converted to A $\beta$ -PET $^+$  than in those who remained A $\beta$ -PET $^-$  over the five-year follow-up period ( $n = 75$ ) (**Fig. 1A**). No significant differences were observed between these two groups concerning

**Table 1**  
Baseline characteristics of the study population $^a$ .

	Whole population ( $n = 200$ )	High plasma A $\beta$ 42/A $\beta$ 40 ( $n = 135$ ) $^\dagger$	Low plasma A $\beta$ 42/A $\beta$ 40 ( $n = 65$ ) $^\ddagger$	$P$ -value
<i>Demographics</i>				
Age, years	67.0 [60.0–70.0]	64.0 [60.0–69.0]	69.0 [65.0–73.0]	<b>0.001</b>
Female, $n$ (%)	126 (63.0)	95 (70.4)	31 (47.7)	<b>0.002</b>
<i>APOE <math>\epsilon</math>4, <math>n</math> (%)</i>				
0 alleles	148 (74.0)	110 (81.5)	38 (58.5)	<b>0.002</b>
1 allele	47 (23.5)	23 (17.0)	24 (36.9)	
2 alleles	5 (2.5)	2 (1.5)	3 (4.6)	
Education, years	15.0 [11.0–18.0]	15.0 [12.0–18.0]	16.0 [10.0–18.0]	<b>0.995</b>
<i>Neuroimaging</i>				
A $\beta$ -PET, CL	–1.69 [–6.70–8.21]	–3.70 [–7.92–1.69]	11.39 [–2.17–35.37]	<b>&lt;0.001</b>
<i>A<math>\beta</math>-PET positivity</i>				
A $\beta$ -PET $^-$ , $n$ (%)	164 (82)	130 (96.3)	34 (52.3)	<b>&lt;0.001</b>
A $\beta$ -PET $^+$ , $n$ (%)	36 (18)	5 (3.7)	31 (47.7)	
Hippocampal volume $^{\ddagger,\S}$ , mm $^3$	3,606.1 [3,401.2–3,820.3]	3,619.2 [3,454.1–3,822.0]	3,603.6 [3,312.1–3,769.0]	0.181
Ventricular volume $^{\ddagger,\S}$ , mm $^3$	25,554.0 [20,116.6–33,576.8]	24,319.2 [18,727.2–32,028.2]	28,119.5 [22,732.5–34,933.1]	<b>0.049</b>
Cortical volume $^{\ddagger,\S}$ , mm $^3$	420,841.3 [407,841.9–438,278.7]	421,433.2 [405,119.4–438,379.1]	420,623.5 [408,830.9–437,630.1]	0.737
<i>Plasma biomarkers</i>				
Plasma A $\beta$ 40, pg/mL	273.6 [248.9–300.2]	267.4 [244.0–292.3]	287.2 [263.8–309.0]	<b>0.004</b>
Plasma A $\beta$ 42, pg/mL	69.5 [62.1–76.7]	72.3 [65.8–79.3]	62.0 [56.2–68.6]	<b>&lt;0.001</b>
Plasma A $\beta$ 42/A $\beta$ 40	0.257 [0.234–0.276]	0.268 [0.257–0.283]	0.219 [0.205–0.232]	<b>&lt;0.001</b>
<i>Global cognition</i>				
MMSE, score	29.5 [29.0–30.0]	29.0 [29.0–30.0]	30.0 [29.0–30.0]	0.119

Abbreviations: APOE apolipoprotein E, CL centiloid, PET positron emission tomography, MMSE Mini-Mental State Examination.

$^a$  : Data are median [IQR] values, except for the variable "female", "APOE" and "A $\beta$ -PET positivity", which are the number of cases (%). Differences between groups were tested using Mann-Whitney U test, Chi-square tests or Fisher's exact test, as appropriate.

$^\dagger$  : High and low plasma A $\beta$ 42/A $\beta$ 40 was defined using a cutoff of 0.241.

$^\ddagger$  : Whole population ( $n = 198$ ); High A $\beta$ 42/A $\beta$ 40 ( $n = 133$ ); Low A $\beta$ 42/A $\beta$ 40 ( $n = 65$ ).

$^\S$  : Data correspond to regional volume corrected by total intracranial volume.

**Table 2**  
Rates of A $\beta$ -PET positivity, all-cause MCI, and MCI due to AD conversion.

<i>A<math>\beta</math>-PET converters</i>				
	A $\beta$ -PET- at V0 (n = 164)	High plasma A $\beta$ 42/A $\beta$ 40 (n = 130)*	Low plasma A $\beta$ 42/A $\beta$ 40 (n = 34)*	P-value
Withdrawals, n (%)	26 (15.9)	19 (14.6)	7 (20.6)	<b>0.015</b>
Converters, n (%)	24 (14.6)	15 (11.5)	9 (26.5)	
Non-converters, n (%)	114 (69.5)	96 (73.9)	18 (52.9)	
<i>All-cause MCI converters</i>				
	Whole population (n = 200)	High plasma A $\beta$ 42/A $\beta$ 40 (n = 135)*	Low plasma A $\beta$ 42/A $\beta$ 40 (n = 65)*	P-value
Withdrawals, n (%)	7 (3.5)	4 (3.0)	3 (4.6)	0.156
Converters, n (%)	44 (22.0)	26 (19.2)	18 (27.7)	
Non-converters, n (%)	149 (74.5)	105 (77.8)	44 (67.7)	
<i>MCI due to AD converters</i>				
	Whole population (n = 200)	High plasma A $\beta$ 42/A $\beta$ 40 (n = 135)*	Low plasma A $\beta$ 42/A $\beta$ 40 (n = 65)*	P-value
Withdrawals, n (%)	27 (13.5)	19 (14.1)	8 (12.3)	<b>&lt;0.001</b>
Converters, n (%)	23 (11.5)	7 (5.2)	16 (24.6)	
Non-converters, n (%)	150 (75.0)	109 (80.7)	41 (63.1)	

Data are number of cases (%). Differences between groups were tested using Chi-square test.

\* : High and low plasma A $\beta$ 42/A $\beta$ 40 was defined using a cutoff of 0.241.

demographic variables (age, sex, or education) or APOE  $\epsilon$ 4 status. However, as expected, converters already had higher A $\beta$ -PET CL values at baseline, in comparison to non-converters (median [IQR]: 3.87 [0.618–8.15] vs  $-4.39$  [ $-8.94$  to  $-0.57$ ] CL;  $P < 0.001$ ).

With regard to the subsample who underwent A $\beta$ -PET scanning at visit 5 ( $n = 119$ ), a statistically significant correlation was found between lower baseline A $\beta$ 42/A $\beta$ 40 and higher cortical amyloid deposition quantified as CL increments after 5 years (Spearman  $\rho = -0.362$ ;  $P < 0.001$ ) (Fig. 1B). Furthermore, when participants were divided into quartiles based on their baseline A $\beta$ 42/A $\beta$ 40 values, those in the lowest quartile (Q1) accumulated more cortical amyloid than subjects in the other three quartiles. These differences were only statistically significant when comparing Q1 and Q4 ( $P = 0.002$ ) (Fig. 1C), but a progressive amyloid accumulation profile was found throughout the four quartiles.

During the study, 9 of 34 (26.5 %) individuals with low plasma ratio progressed to A $\beta$ -PET+, while only 15 of 130 (11.5 %) individuals with high plasma ratio did ( $P = 0.015$ ; Table 2). The higher cumulative probability of converting to amyloid-positivity in the low A $\beta$ 42/A $\beta$ 40 group was shown in the Kaplan-Meier analysis (Log-rank test:  $P = 0.005$ ) (Fig. 1D).

Finally, Cox proportional-hazards models also revealed that low plasma A $\beta$ 42/A $\beta$ 40 ratio was associated with an increased risk of future progression to A $\beta$ -PET+ (HR = 3.13, 95 % CI 1.35–7.25,  $P = 0.008$ ). This association remained statistically significant after adjusting for age and APOE  $\epsilon$ 4 status (HR = 2.84, 95 % CI 1.14–7.04,  $P = 0.025$ ).

### 3.3. Association between baseline A $\beta$ 42/A $\beta$ 40 and progression to all-cause MCI and MCI due to AD at 5-year follow-up

All 200 individuals enrolled in the FACEHBI study met the diagnosis criteria for SCD at baseline. During the follow-up period, a total of 44 individuals progressed to all-cause MCI (Table 2): 10 in V1, 11 in V2, 8 in V3, 7 in V4, and 8 in V5. At baseline, individuals who later progressed to all-cause MCI were older (median [IQR]: 70.0 [67.0–73.0] vs 63.0 [59.0–68.0] years;  $P < 0.001$ ), had lower educational level (14.0 [9.3–16.0] vs 16.0 [13.0–20.0] years;  $P = 0.003$ ) and exhibited higher baseline A $\beta$ -PET values (3.23 [ $-3.72$ –29.73] vs  $-3.49$  [ $-7.74$ –3.28] CL;  $P < 0.001$ ) than those who remained as SCD during the whole follow-up. All-cause MCI converters only had slightly lower A $\beta$ 42/A $\beta$ 40 values at baseline than stable SCD (0.255 [0.212–0.270] vs 0.259 [0.242–0.279];

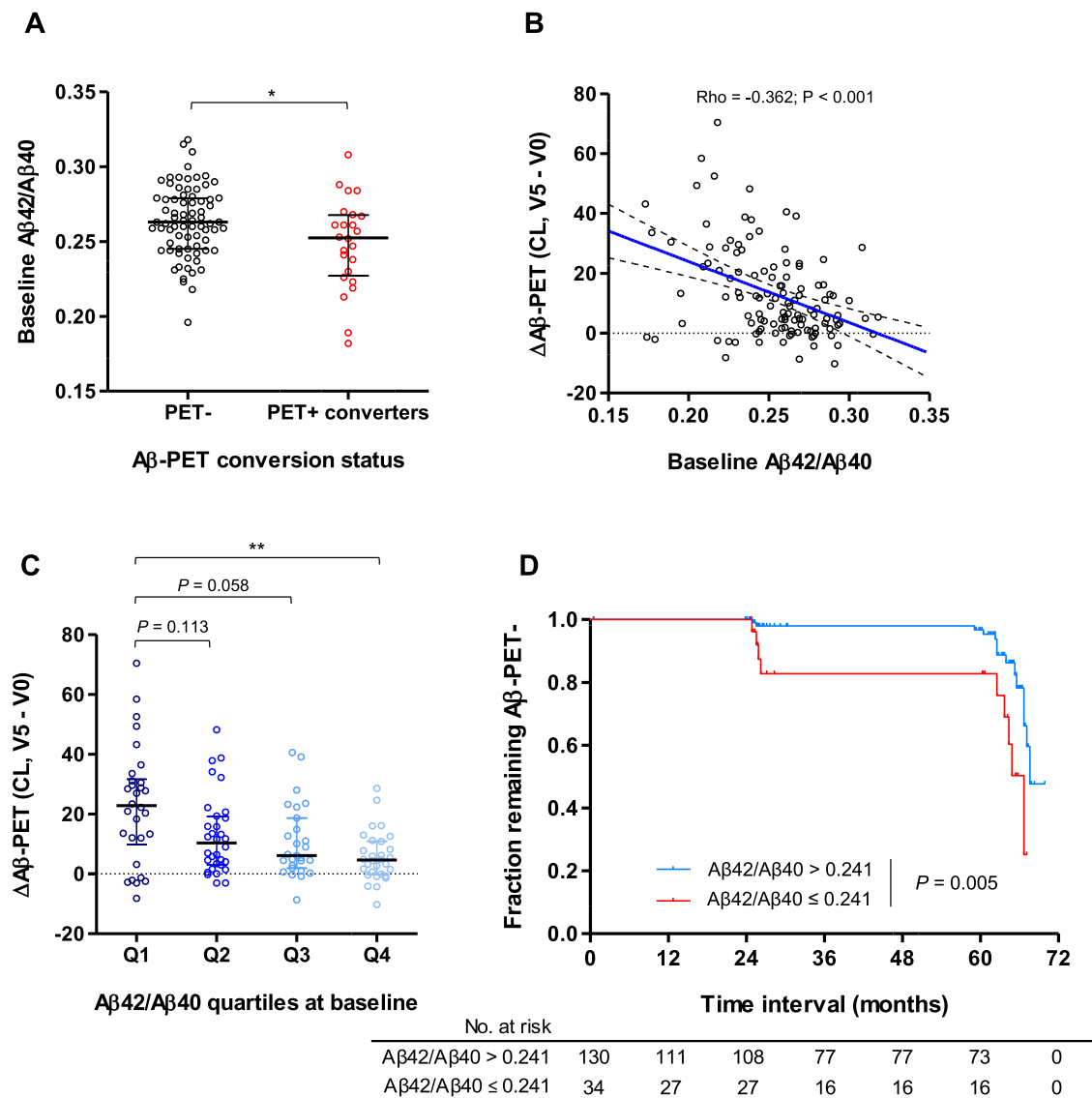
$P = 0.054$ ) (Supplementary Fig. 1A).

The low A $\beta$ 42/A $\beta$ 40 group did not progress to all-cause MCI more frequently ( $P = 0.156$ ) (Table 2) than the high A $\beta$ 42/A $\beta$ 40 group. Additionally, they did not show a significantly increased risk of progression, either in the Kaplan-Meier analysis (Log-rank test:  $P = 0.075$ ) (Supplementary Fig. 1B), or in the unadjusted (HR = 1.72, 95 % CI 0.94–3.13,  $P = 0.079$ ) and adjusted (HR = 1.12, 95 % CI 0.58–2.17,  $P = 0.738$ ) Cox regression analyses. Within this group of converters, 23 of the 44 individuals (52 %) progressed to MCI due to AD at the end of the follow-up (Table 2). Of these, 18 entered the study as A $\beta$ -PET+ and only 5 of them progressed to both, MCI and A $\beta$ -PET positivity.

Those participants who converted to MCI due to AD over the study (defined as MCI with A $\beta$ -PET+) were found to be significantly older (median [IQR]: 70.0 [69.0–73.0] vs 64.0 [59.0–68.0] years;  $P < 0.001$ ) and more frequently carriers of APOE  $\epsilon$ 4 (61 % vs 22 %,  $P < 0.001$ ). They also exhibited higher cortical amyloid burden (26.39 [19.32–58.68] vs  $-2.50$  [ $-7.06$ –3.16] CL;  $P < 0.001$ ) and lower A $\beta$ 42/A $\beta$ 40 (0.213 [0.195–0.253] vs 0.260 [0.241–0.278];  $P < 0.001$ ) than the non-converters (those who remained as SCD or those who progressed to MCI, but remained A $\beta$ -PET-) (Fig. 2A).

In Fig. 2B, the participants were split according to diagnosis (SCD vs MCI) and A $\beta$ -PET status (+/-) at V5. Baseline A $\beta$ 42/A $\beta$ 40 values were lower in the A $\beta$ -PET+ group than in the A $\beta$ -PET- group, both in the SCD ( $P = 0.008$ ) and the MCI populations ( $P = 0.002$ ). Additionally, those who converted to MCI due to AD had a significantly lower A $\beta$ 42/A $\beta$ 40 ratio at baseline than the non-converter subgroup SCD and A $\beta$ -PET- ( $P < 0.001$ ). No statistically significant differences were found between both A $\beta$ -PET+ groups (SCD and MCI) after correcting for multiple comparisons ( $P = 0.176$ ). Note that the A $\beta$ 42/A $\beta$ 40 ratio was not decreased in the MCI and A $\beta$ -PET- group (other-cause MCI).

Throughout the study, 16 out of 65 (24.6 %) participants in the low A $\beta$ 42/A $\beta$ 40 group progressed to MCI due to AD, while only 7 out of 135 (5.2 %) progressed in the high A $\beta$ 42/A $\beta$ 40 group ( $P < 0.001$ ; Table 2). Additionally, low baseline A $\beta$ 42/A $\beta$ 40 ratio was associated with a significantly higher conversion rate to MCI due to AD over time, as shown by the Kaplan-Meier analysis (Log-Rank Test:  $P < 0.001$ ; Fig. 2C). This association was further supported by both unadjusted (HR = 6.68, 95 % CI 2.61–17.10,  $P < 0.001$ ) and adjusted Cox models (HR = 3.25, 95 % CI 1.17–9.01,  $P = 0.024$ ).



**Fig. 1.** Association between baseline Aβ42/Aβ40 ratio and amyloid pathology at 5-year follow-up. **A.** Distribution of Aβ42/Aβ40 ratio at baseline between stable Aβ-PET- at V5 and converters to Aβ-PET+ during the whole follow-up period. Horizontal lines depict medians and whiskers depict interquartile ranges. Plasma Aβ42/Aβ40 values were compared between the two groups using Mann-Whitney U test. \**P* < 0.05. **B.** Correlation between plasma Aβ42/Aβ40 at baseline and amyloid accumulation at 5-year follow-up visit, as determined by Aβ-PET CL increments (V5 - V0). Solid blue line represents the regression line; dashed lines represent 95 % confident interval. **C.** Distribution of Aβ-PET CL increments among the quartiles of plasma Aβ42/Aβ40 at baseline. Horizontal lines depict medians and whiskers depict interquartile ranges. Aβ-PET CL increments among quartiles were compared using Mann-Whitney U test with Bonferroni correction applied to adjust for multiple comparisons. \*\**P* < 0.01. **D.** Kaplan-Meier curves showing the fraction of participants remaining Aβ-PET-. Vertical tick marks on lines indicate times at which the participants were censored. The *P*-value of the Log-rank test is depicted. The table below the graph includes the population at risk of conversion at each timepoint.

**3.4. Association between baseline Aβ42/Aβ40 and brain atrophy at 5-year follow-up**

Individuals in the low Aβ42/Aβ40 group at baseline exhibited a significantly higher reduction of hippocampal (*P* = 0.019) (Supplementary Fig. 2A) and cortical (*P* = 0.023) (Supplementary Fig. 2B) volumes over the course of the study than those in the high Aβ42/Aβ40 group. In addition, the low group also showed significantly greater increases in ventricular volume (*P* = 0.001) (Supplementary Fig. 2C).

**3.5. Association between baseline Aβ42/Aβ40 ratio and cognitive changes at 5-year follow-up**

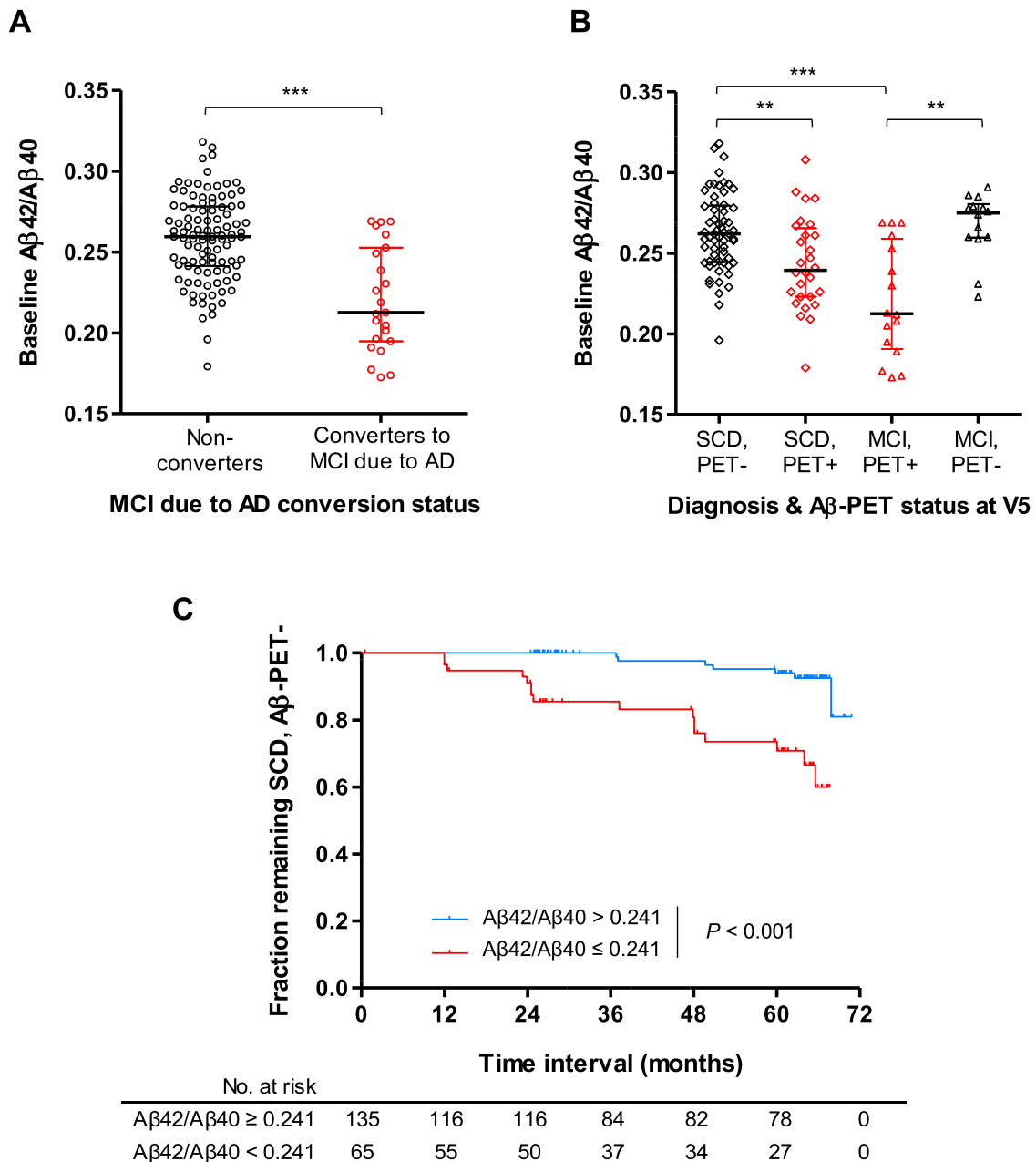
The change in MMSE and cognitive composites scores between the 5-year follow-up and the baseline visits (V5 - V0) is represented in

**Supplementary Fig. 3A-K.** MMSE scores declined more over the follow-up period in the low Aβ42/Aβ40 group compared to the high Aβ42/Aβ40 group (*P* = 0.024). No statistically significant differences were found in the cognitive composites.

**3.6. Longitudinal assessment of brain amyloid deposition, cerebral volume reduction, and cognitive decline using linear mixed-effects models**

- Association between plasma Aβ42/Aβ40 and cortical amyloid deposition

The association between baseline dichotomized plasma Aβ42/Aβ40 ratio groups and cortical amyloid deposition rate was assessed using LMEMs (Fig. 3A). Individuals in the low Aβ42/Aβ40 group accumulated 0.257 CLs more per month (3 CL/year), than those individuals in the



**Fig. 2.** Association between baseline  $A\beta_{42}/A\beta_{40}$  ratio and conversion to MCI due to AD.

**A.** Distribution of  $A\beta_{42}/A\beta_{40}$  at baseline between non-converters at V5 and converters to MCI due to AD during the whole follow-up. The horizontal lines depict the median and the whiskers depict the interquartile ranges. Plasma  $A\beta_{42}/A\beta_{40}$  was compared between groups using the Mann-Whitney U test.  $***P < 0.001$ . **B.** Distribution of  $A\beta_{42}/A\beta_{40}$  at baseline between  $A\beta$ -PET- and  $A\beta$ -PET+ subjects in SCD and MCI groups at 5-year follow-up. The  $A\beta$ -PET+ groups included subjects who were enrolled in the study as  $A\beta$ -PET+ and subjects who converted during follow-up. The horizontal lines depict the medians and the whiskers depict the interquartile ranges. Plasma  $A\beta_{42}/A\beta_{40}$  was compared among groups using the Mann-Whitney U test with Bonferroni correction applied to adjust for multiple comparisons.  $**P < 0.01$ ;  $***P < 0.001$ . **C.** Kaplan-Meier curves showing fraction of individuals remaining SCD and  $A\beta$ -PET-. Vertical tick marks on lines indicate times at which the participants were censored. The  $P$ -value of Log-rank test is depicted. The table below the graph includes the population at risk of conversion at each timepoint.

high  $A\beta_{42}/A\beta_{40}$  group ( $\beta = 0.257$ , 95 % CI 0.177–0.336,  $P < 0.001$ ). The rate of amyloid accumulation was more than three times higher in the low  $A\beta_{42}/A\beta_{40}$  group ( $\beta = 0.372$ , 95 % CI 0.306–0.438) compared to the high  $A\beta_{42}/A\beta_{40}$  group ( $\beta = 0.115$ , 95 % CI 0.071–0.159).

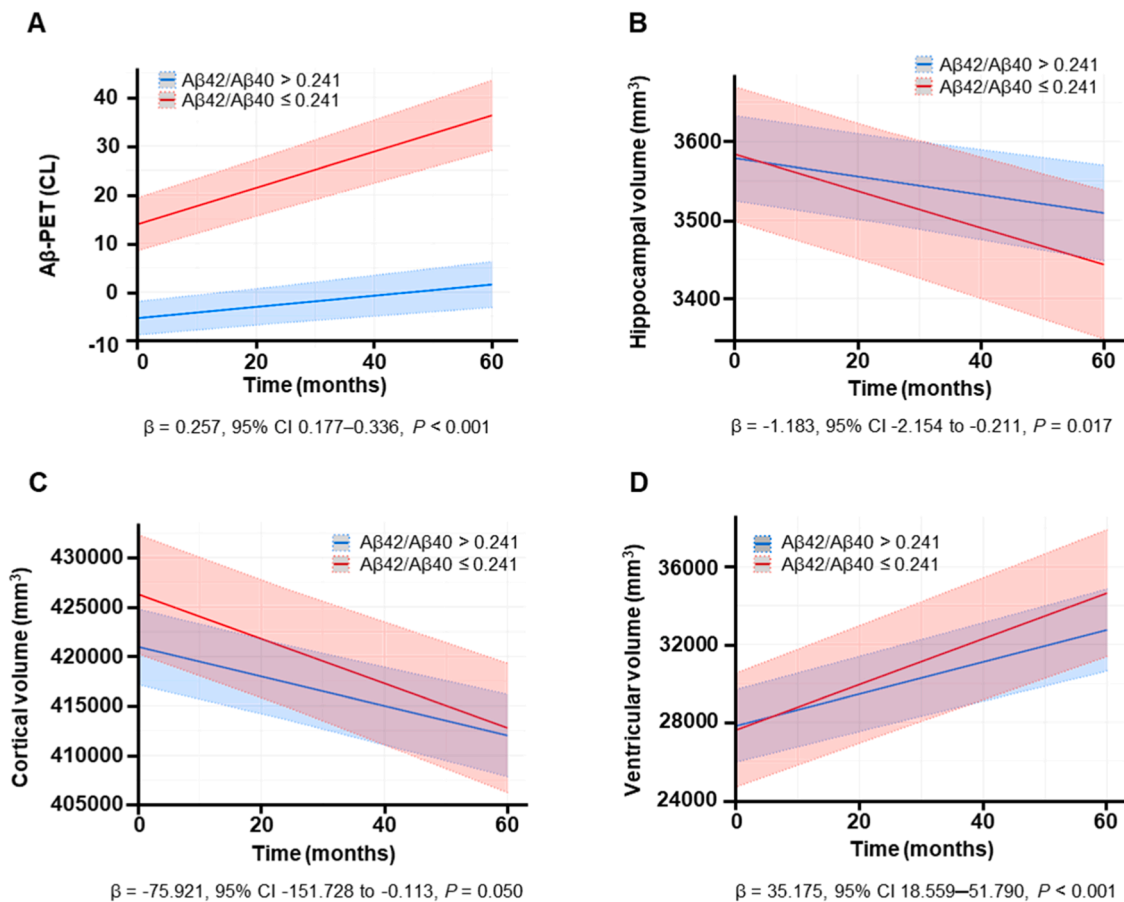
- Association between plasma  $A\beta_{42}/A\beta_{40}$  and changes in brain volume

The relationship between the baseline  $A\beta_{42}/A\beta_{40}$  ratio and longitudinal changes in brain volume was also assessed using LMEMs. The

dichotomized baseline plasma  $A\beta_{42}/A\beta_{40}$  ratio was significantly associated with hippocampal ( $\beta = -1.183$ , 95 % CI  $-2.154$  to  $-0.211$ ,  $P = 0.017$ ) (Fig. 3B) and cortical ( $\beta = -75.921$ , 95 % CI  $-151.728$  to  $-0.113$ ,  $P = 0.050$ ) (Fig. 3C) volume loss, as well as ventricular volume increase ( $\beta = 35.175$ , 95 % CI 18.559–51.790,  $P < 0.001$ ) (Fig. 3D).

- Association between plasma  $A\beta_{42}/A\beta_{40}$  ratio and cognitive decline

Regarding the association between baseline  $A\beta_{42}/A\beta_{40}$  levels in plasma and longitudinal cognitive decline assessed with LMEMs, neither



**Fig. 3.** Prediction of longitudinal amyloid accumulation and brain volume changes.

**A.** Longitudinal amyloid-PET accumulation. **B-C-D:** Longitudinal changes in hippocampal, cortical and ventricular volumes respectively. The average regression line for each group (low baseline Aβ42/Aβ40, in red and high baseline Aβ42/Aβ40, in blue) was plotted from LMEMs including age and APOE ε4 status as covariates. The shaded area represents the 95 % CI. Below each graph, the β coefficient, 95 % CI, and P-value from the corresponding LMEM are included, reflecting the “Aβ42/Aβ40 × time” interaction effect. Abbreviations: Aβ-PET: amyloid-β positron emission tomography. CL: centiloids. CI: confidence interval.

the MMSE nor the ten cognitive composites scores showed a significant longitudinal association with baseline Aβ42/Aβ40 ratio when this biomarker was included in the model in a dichotomized format. However, MMSE and Memory Composite S-FNAME-Occupations showed a trend ( $P = 0.072$  and  $P = 0.101$ , respectively). Indeed, when Aβ42/Aβ40 ratio was included as a continuous variable in these models, significant differences were achieved (MMSE:  $\beta = 0.146, 95\% \text{ CI } 0.008-0.284, P = 0.038$ ; Memory Composite S-FNAME-Occupations:  $\beta = 0.088, 95\% \text{ CI } 0.013-0.163, P = 0.021$ ).

#### 4. Discussion

The plasma Aβ42/Aβ40 ratio is an early biomarker in the AD continuum, but its potential to predict subsequent pathological features remains uncertain. In the present study, this biomarker, measured using a sensitive method such as ABtest-MS, was shown to be associated with an increased risk of future amyloid accumulation, brain atrophy, and conversion to MCI due to AD in a population of individuals with SCD.

Building on previous analyses from the FACEHBI cohort, this study expands earlier work by examining how baseline plasma Aβ42/Aβ40 relates to subsequent biological and clinical trajectories over five years. While Pascual-Lucas et al., 2023 [15] focused on diagnostic performance and short-term associations, the present study adopts a broader longitudinal perspective and includes a wider set of downstream outcomes. Although the analytic and diagnostic performance of ABtest-MS has already been validated with external cohorts [37,38] and real-world clinical samples [39], its long-term prognostic utility had not been

evaluated in an extensively characterized SCD cohort. By integrating extended longitudinal imaging, clinical, and cognitive endpoints, this work provides the most comprehensive assessment to date of the prognostic value of ABtest-MS-derived Aβ42/Aβ40 in a very early stage of AD.

In this longitudinal study, baseline Aβ42/Aβ40 ratio was already decreased in those individuals who later converted to Aβ-PET positivity, regardless of their diagnosis 5 years later (SCD vs MCI). In addition, this biomarker was associated with an increased rate of brain amyloid deposition over five years and consequently, with a higher risk of conversion to Aβ-PET positivity. These findings suggest that the Aβ42/Aβ40 ratio may be a useful biomarker for assessing future amyloid accumulation in individuals at risk of developing AD dementia.

Other groups exploring this same association have reached similar conclusions. In a cohort of mostly CH participants, Schindler et al. found that individuals with a positive plasma Aβ42/Aβ40 ratio at baseline had a higher risk of conversion to amyloid-PET+ than those with a negative plasma Aβ42/Aβ40 [40]. In CH participants from the BIOFINDER-2, Knight AD and BIOFINDER-1 cohorts, Janelidze et al. reported that lower Aβ42/Aβ40 ratio was associated with higher baseline Aβ-PET CL values and showed a significant correlation with increasing Aβ-PET load over time [23]. Finally, Pereira et al. concluded that the plasma Aβ42/Aβ40 ratio was the only biomarker independently associated with progressive global amyloid accumulation over time in non-demented individuals [21].

Plasma p-tau217, another highly accurate biomarker for amyloid deposition, has also demonstrated strong predictive value for cortical

amyloid accumulation, even in CH individuals [41]. Furthermore, earlier findings have shown that combining plasma A $\beta$ 42/A $\beta$ 40 ratio with p-tau217 improves both the detection [42] and progression [23] of A $\beta$  pathology. In addition, to date, the only FDA-approved blood test for the early detection of amyloid plaques associated with AD combines p-Tau217 and A $\beta$ 42. Based on this, future research should explore whether adding p-tau217 measurements may further improve the ability of our model to capture amyloid-related changes, although the diagnostic accuracy of this biomarker is highest in symptomatic individuals compared with cognitively unimpaired populations [43].

Previous studies have investigated the role of plasma biomarkers in predicting the future progression from MCI to AD [44,45]. However, the potential of the plasma A $\beta$ 42/A $\beta$ 40 ratio to provide prognostic information regarding future clinical changes in CH individuals has been less explored. Some studies have described that a lower A $\beta$ 42/A $\beta$ 40 ratio at baseline is associated with a higher risk of subsequent development of MCI or AD dementia in CH or SCD individuals [22,46]. However, Shen and colleagues did not find any difference between the A+T-N- (Amyloid/Tau/Neurodegeneration) and the A-T-N- groups, when amyloid pathology was identified with plasma A $\beta$ 42/A $\beta$ 40 [47].

In the present study, the plasma A $\beta$ 42/A $\beta$ 40 ratio was not significantly associated with conversion to all-cause MCI. These results may be explained, as the A $\beta$ 42/A $\beta$ 40 ratio is a biomarker of amyloid pathology, and mixed pathologies, some of them unrelated to A $\beta$ , may be present in this cohort, as not all individuals who converted to MCI had an A $\beta$ -PET+ scan. Diagnostic aid tools designed to identify brain amyloid deposition, such as ABtest-MS, are not expected to have high accuracy when applied to non-AD cases (other-cause MCI), where amyloid pathology is absent. For this reason, we focused our analysis on assessing the conversion to MCI due to AD (MCI with A $\beta$ -PET+). In this context, SCD individuals with a low A $\beta$ 42/A $\beta$ 40 ratio at baseline showed a higher risk of conversion to MCI, even after adjusting for age and APOE  $\epsilon$ 4 status. Baseline A $\beta$ 42/A $\beta$ 40 values were significantly lower in this group than in the stable A- participants (SCD with A $\beta$ -PET-). The results of the present study suggest that the plasma A $\beta$ 42/A $\beta$ 40 ratio, measured using an accurate and robust method, is associated with the progression from SCD to MCI due to AD. Moreover, the very high negative predictive value observed in this cohort indicates that individuals with high baseline ratio values—predominantly corresponding to A $\beta$ -PET-negative cases (true negatives)—show a very low risk of conversion to MCI due to AD. By contrast, individuals classified as positive (low A $\beta$ 42/A $\beta$ 40 ratio) warrant close follow-up, as data indicate that they are at increased risk of conversion.

Regarding other plasma biomarkers, some studies have reported that plasma p-tau isoforms, particularly p-tau181 [48] and p-tau217 [22,25], are also associated with an increased risk of progression to AD dementia in cognitively healthy individuals.

The association between baseline A $\beta$ 42/A $\beta$ 40 values and longitudinal changes in brain MRI parameters was subsequently assessed. Our findings suggest that the A $\beta$ 42/A $\beta$ 40 ratio is associated with early neurodegenerative changes occurring in the initial stages of AD. After two years of follow-up, an association between baseline A $\beta$ 42/A $\beta$ 40 levels and changes in ventricular volume had been previously described [15]. This trend persisted in the 5-year follow-up analysis, where participants with lower A $\beta$ 42/A $\beta$ 40 ratios showed greater increases in ventricular volume, and greater decreases in hippocampal and cortical volume. In addition, LMEMs indicated that individuals with lower A $\beta$ 42/A $\beta$ 40 levels experienced significantly faster rates of change in hippocampal, cortical and ventricular volumes.

Recently, Mitolo *et al.* published a review addressing the association between blood-based biomarkers and brain MRI parameters across the clinical AD continuum [24]. However, few longitudinal studies in CH populations have been published so far. Dark *et al.* did not find any association between baseline A $\beta$ 42/A $\beta$ 40 values and changes in brain volume in a population of CH individuals [49]. However, Simrén *et al.* reported that lower A $\beta$ 42 and A $\beta$ 42/A $\beta$ 40 at baseline were related to

grey matter loss in the orbitofrontal cortex ( $P < 0.05$ ). In addition, within the CH group, they found an association between longitudinal changes of these biomarkers and grey matter volume change in the posterior cingulate and prefrontal cortex [50]. Thus, together with some others, our results highlight the association between plasma A $\beta$ 42/A $\beta$ 40 ratio values and longitudinal brain volumetric changes in individuals at the early stages of AD, even in preclinical AD.

Other plasma biomarkers such as p-tau181, p-tau217, NfL or GFAP (glial fibrillary acidic protein) have also shown associations with different MRI outcome measures [21,24,49]. However, due to heterogeneity in the findings, further research is still needed in preclinical populations.

In the present study, predictive models were developed to explore the trajectory of MMSE scores and ten cognitive composite measures. Significant associations were found for MMSE and the Memory Composite S-FNAME Occupations, when the ratio was included as a continuous variable in the model. However, only a trend was obtained when the ratio was included in a dichotomized format. In a cohort of older adults with subjective memory concerns, Giudici *et al.*, found that low A $\beta$ 42/A $\beta$ 40 was related to greater MMSE decline over time [51]. However, some other groups studying this issue in CH populations did not find a significant association with MMSE, even when employing different methodologies to measure the A $\beta$ 42/A $\beta$ 40 ratio [44,49,52,53]. This may be explained by the potentially limited sensitivity of this test to detect subtle changes in the CH population, leading to increased variability [54]. Thus, maybe, this association might have shown a strongest effect with PACC (Preclinical Alzheimer Cognitive Composite) [22], as this test was created to specifically detect early AD-related changes in non-demented individuals [22,55]. In any case, other studies did not find an association between plasma A $\beta$ 42/A $\beta$ 40 and PACC scores [52, 53] over time. The significant results found in our study for the Memory Composite S-FNAME Occupations may reflect an association between the plasma A $\beta$ 42/A $\beta$ 40 ratio and early worsening in complex associative memory performance, which is a cognitive endophenotype closely linked to early AD.

The heterogeneity in study design, and variability in the neuropsychological tests and composite measures used, complicates direct comparisons between studies. However, some groups have obtained promising results in assessing the ability of plasma A $\beta$ 42/A $\beta$ 40 to predict future cognitive decline in CH populations. Lim *et al.* showed that higher plasma A $\beta$  composite scores (generated by combining APP<sub>669-711</sub>/A $\beta$ 1-42 and A $\beta$ 1-40/A $\beta$ 1-42), measured using IP-MS, were moderately related to accelerated decline in both episodic memory and executive functions [56]. Giudici *et al.* and Aschenbrenner *et al.* reported that lower values of A $\beta$ 42/A $\beta$ 40, measured with IP-LC/MS, were associated with a faster decline in cognitive performance, measured by multiple outcomes or a global cognitive composite [51,57]. Verberk *et al.* also reported that lower plasma A $\beta$ 42/A $\beta$ 40 levels were associated with a steeper rate of cognitive worsening on attentional, memory, and executive, but not language, test performances [58]. All these results highlight the potential role of A $\beta$ 42/A $\beta$ 40 to predict future decline in cognition, although further research is needed in early AD populations, given the conflicting findings reported so far [59].

Plasma p-tau217 has also been described to be associated with cognitive worsening [21,25]. Regarding plasma NfL and GFAP, the literature shows mixed results concerning their potential as predictors of cognitive decline in CH populations [59].

Several strengths of this study can be listed. First, the use of a population of SCD individuals provides a unique opportunity for timely interventions, potentially delaying or preventing further cognitive decline. Second, an accurate antibody-free MS-method was used to quantify A $\beta$ 42/A $\beta$ 40 in plasma, a method that has proven reliable in terms of accuracy and precision [15]. Third, the use of the A $\beta$ 42/A $\beta$ 40 ratio offers a key methodological advantage over other individual plasma biomarkers such as pTau, NfL or GFAP, as it is less affected by comorbidities such as renal dysfunction or body mass index, effectively

compensating for influences that impact the individual peptides [60, 61]. Fourth, each study participant was recruited from the same clinic and all blood samples were processed and analyzed in the same laboratory. Therefore, the effect of pre-analytical and analytical variability that may affect plasma A $\beta$  levels was minimized. Fifth, all the procedures (sample collection, PET scan, MRI scan, and cognitive assessment) were conducted within a short period (3 months) during the same visit. Finally, the association between A $\beta$ 42/A $\beta$ 40 and cognitive changes was assessed using multiple clinical outcomes.

## 5. Limitations

In this study, we focused on the potential utility of a single plasma biomarker. However, as mentioned above, incorporating additional biomarkers could provide valuable insights into how they compare or complement each other in evaluating risk among SCD individuals for developing AD.

The FACEHBI cohort is highly characterized, but its size may limit the generalizability of the findings to wider populations. Validation of these results in an independent cohort would provide valuable additional evidence, further supporting previous work obtained using ABtest-MS, in diverse ethnic and diagnostic groups [37,38]. Another limitation of this study is that the cohort used may not fully represent the diversity of the general population. Real-world studies or community-based cohorts, with a broader variety in terms of demographic characteristics, lifestyles and comorbidities, should also be implemented to estimate the real potential of plasma biomarkers [62]. Finally, further studies are needed to optimize and personalize disease predictions in clinical practice.

In conclusion, the findings of this study suggest that the plasma A $\beta$ 42/A $\beta$ 40 ratio could serve as a valuable biomarker associated with longitudinal future amyloid accumulation, brain atrophy and conversion to MCI due to AD in individuals with SCD. Beyond indicating the potential onset of objective cognitive decline, this marker provides insights into disease-related processes in this population. Therefore, the plasma A $\beta$ 42/A $\beta$ 40 ratio could contribute to stratifying individuals by risk, facilitating earlier and more personalized interventions.

## Funding

Funds from Ace Alzheimer Center Barcelona, Grifols, Life Molecular Imaging GmbH, Laboratorios Echevarne S.A. and Araclon Biotech support the FACEHBI study.

AR received funding from Spanish Instituto de Salud Carlos III (ISCIII), Acción Estratégica en Salud, integrated in the Spanish National R+D+I Plan and financed by ISCIII Subdirección General de Evaluación and the Fondo Europeo de Desarrollo Regional (FEDER "Una manera de hacer Europa") grants PI13/02434, PI16/01861, PI19/01240, PI19/01301, PI22/00258 and PI22/01403 and the ISCIII national grant PMP22/00022, funded by the European Union (NextGenerationEU); CIBERNED (ISCIII) under the grants CB06/05/2004 and CB18/05/00010; ADAPTED project - European Union/EFPIA Innovative Medicines Initiative Joint (grant numbers 115975); the PREADAPT project - Joint Program for Neurodegenerative Diseases (JPND) grant N° AC19/00097; the HARPONE project, Agency for Innovation and Entrepreneurship (VLAIO) grant N° PR067/21 and Janssen and the DESCARTES project is funded by German Research Foundation (DFG).

MB received funding from CIBERNED (Instituto de Salud Carlos III (ISCIII); EU/EFPIA Innovative Medicines Initiative Joint Undertaking, ADAPTED Grant No. 115975; EXIT project, EU Euronanomed3 Program JCT2017 Grant No. AC17/00100; MOPEAD, Innovative Medicine Initiative, Grant. N°. 115985; PreDADQoL, ERA-NET (call 2015). Grant n° AC15/00082; TARTAGLIA (Red federada para acelerar la aplicación de la inteligencia artificial en el sistema sanitario español); PREADAPT project, Joint Program for Neurodegenerative Diseases (JPND) Grant No. AC19/00097; GECONEU Grant No. 2023-1-ELO1-KAZZO-HED-

000032173 co-funded by the European Union; Grants PI13/02434, PI16/01861, BA19/00020, and PI19/01301 from the Acción Estratégica en Salud, integrated in the Spanish National RDCI Plan and financed by Instituto de Salud Carlos III (ISCIII)- Subdirección General de Evaluación and the Fondo Europeo de Desarrollo Regional (FEDER - "Una manera de Hacer Europa"); Fundació "La Caixa" and Grifols (GR@ACE project); and Proyectos de Investigación de Medicina Personalizada (ISCIII), PMP-DEGESCO, Grant N° PMP22/00022.

MA received funding from the Spanish Instituto de Salud Carlos III (ISCIII) Acción Estratégica en Salud, integrated in the Spanish National R+D+I Plan and financed by ISCIII Subdirección General de Evaluación and the Fondo Europeo de Desarrollo Regional (FEDER "Una manera de hacer Europa") grant PI22/01403.

MM received funding from the from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no. 796706 and the Instituto de Salud Carlos III (ISCIII) Acción Estratégica en Salud, integrated in the Spanish National RDCI Plan and financed by ISCIII-Subdirección General de Evaluación and the Fondo Europeo de Desarrollo Regional (FEDER - Una manera de hacer Europa) grant PI19/00335.

## Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethical standards

All participants gave written informed consent according to the principles of the Declaration of Helsinki. The FACEHBI and FACEHBI-2 study protocols (which included visits 0, 1 and 2, and visits 3, 4 and 5, respectively) were approved by the ethics committee of the Hospital Clínic i Provincial (Barcelona, Spain).

## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used AI tool based on large language models (LLMs) in order to improve language and clarity of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication. The use of AI-assisted technologies was limited to language refinement and involved less than 15 % of the overall writing process.

## CRedit authorship contribution statement

**Noelia Fandos:** Writing – original draft, Visualization, Supervision, Investigation, Formal analysis, Data curation. **María Pascual-Lucas:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Leticia Sarasa:** Writing – review & editing, Methodology, Investigation, Data curation. **Jose Terencio:** Writing – review & editing, Supervision, Conceptualization. **M<sup>a</sup> Eugenia Sáez:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Juan Pablo Tartari:** Writing – review & editing, Investigation, Data curation. **Ángela Sanabria:** Writing – review & editing, Investigation, Data curation. **Oscar Sotolongo-Grau:** Writing – review & editing, Investigation, Data curation. **Amanda Cano:** Writing – review & editing, Investigation, Data curation. **Lluís Tàrraga:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Miren Jone Gurruchaga:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Agustín Ruíz:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Xavier Montalban:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Mercè Boada:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Montserrat Alegret:** Writing

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#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Noelia Fandos reports a relationship with Araclon Biotech - Grifols that includes: employment. Maria Pascual-Lucas, Leticia Sarasa, Jose Antonio Allue reports a relationship with Araclon Biotech - Grifols that includes: employment. Jose Terencio reports a relationship with Grifols SA that includes: employment. Juan Pablo Tartari, Angela Sanabria, Oscar Sotolongo-Grau, Amanda Cano, Lluís Tarraga, Miren Jone Guruchaga, Agustin Ruiz, Xavier Montalban, Merce Boada, Montserrat Alegret, Marta Marquíe reports a relationship with ACE Alzheimer Center Barcelona that includes: employment. Merce Boada reports a relationship with Araclon Biotech, Avid, Grifols, Lilly, Nutricia, Roche, Eisai, Servier that includes: consulting or advisory. Merce Boada reports a relationship with Araclon Biotech, Biogen, Grifols, Nutricia, Roche, Servier that includes: speaking and lecture fees. Merce Boada reports a relationship with Abbvie, Araclon, Biogen Research Limited, Bioiberica, Grifols, Lilly, S.A, Merck Sharp & Dohme, Kyowa Hakko Kirin, Laboratorios Servier, Nutricia SRL, Oryzon Genomics, Piramal Imaging Limited, Roche Pharma SA, and Schwabe Farma Iberica SLU that includes: funding grants. Agustin Ruiz reports a relationship with Landsteiner Genmed and Grifols SA that includes: board membership and equity or stocks. Marta Marquíe reports a relationship with Roche Diagnostics Corporation that includes: consulting or advisory. Marta Marquíe reports a relationship with Araclon Biotech - Grifols that includes: board membership. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgments

We are grateful to all FACEHBI participants, without whom this study would not have been possible. We thank all FACEHBI sponsors for making this project possible and all of the investigators from Ace Alzheimer Center Barcelona, Hospital Clinic i Provincial de Barcelona, Clínica Corachan and Life Molecular Imaging GmbH for their close collaboration and continuous intellectual input. The FACEHBI study group:

Aguilera N<sup>1</sup>, Alarcón-Martín E<sup>1</sup>, Alegret M<sup>1,2</sup>, Allué JA<sup>3</sup>, Bayón-Bujan P<sup>1</sup>, Berthier M<sup>4</sup>, Blázquez-Folch J<sup>1</sup>, Boada M<sup>1,2</sup>, Buendía M<sup>1</sup>, Bullich S<sup>5</sup>, Campos F<sup>6</sup>, Calm-Salvans B<sup>1</sup>, Cano A<sup>1,2</sup>, Casales F<sup>1</sup>, Cañabate P<sup>1,2</sup>, Cañada L<sup>1</sup>, Cuevas C<sup>1</sup>, de Rojas I<sup>1</sup>, Diego S<sup>1</sup>, Domingues-Kolinger G<sup>5</sup>, Escudero JM<sup>7</sup>, Espinosa A<sup>1,2</sup>, Fandos N<sup>3</sup>, Fernández MV<sup>1</sup>, Gailhaje-net A<sup>1</sup>, García-González P<sup>1,2</sup>, Giménez J<sup>7</sup>, Gómez-Chiari M<sup>7</sup>, Guitart M<sup>1</sup>, Gurruchaga MJ<sup>1</sup>, Gutiérrez PC<sup>1</sup>, Hernández I<sup>1,2</sup>, Ibarria M<sup>1</sup>, Lafuente A<sup>1</sup>, Lomeña F<sup>6</sup>, Marquíe M<sup>1,2</sup>, Martín E<sup>1</sup>, Martínez C<sup>1</sup>, Martínez M<sup>1</sup>, Miguel A<sup>1</sup>, Moreno M<sup>1</sup>, Morera A<sup>1</sup>, Montreal L<sup>1</sup>, Muñoz N<sup>1</sup>, Muñoz-Morales A<sup>1</sup>, Niñerola A<sup>6</sup>, Nogales AB<sup>1</sup>, Núñez L<sup>8</sup>, Olivé C<sup>1</sup>, Orellana A<sup>1,2</sup>, Ortega G<sup>1,2</sup>, Páez A<sup>8</sup>, Pancho A<sup>1</sup>, Pelejà E<sup>1</sup>, Pérez-Martínez E<sup>5</sup>, Pérez-Cordon A<sup>1</sup>, Pérez-Grijalba V<sup>3</sup>, Pascual-Lucas M<sup>3</sup>, Perissinotti A<sup>6</sup>, Preckler S<sup>1</sup>, Puerta R<sup>1</sup>, Ramis MI<sup>1</sup>, Rodríguez JN<sup>1</sup>, Roé-Vellvé N<sup>5</sup>, Romero J<sup>3</sup>, Rosende-Roca M<sup>1</sup>, Ruiz A<sup>1,2</sup>, Sanabria A<sup>1,2</sup>, Sanz-Cartagena P<sup>1</sup>, Sarasa L<sup>3</sup>, Seguer S<sup>1</sup>, Soliver A<sup>1</sup>, Sotolongo-Grau O<sup>1</sup>, Stephens A<sup>5</sup>, Tartari JP<sup>1</sup>, Tàrraga L<sup>1,2</sup>, Tejero MA<sup>7</sup>, Terencio J<sup>3</sup>, Torres M<sup>8</sup>, Valenzuela A<sup>1</sup>, Valero S<sup>1,2</sup>, Vargas L<sup>1</sup>, Vivas A<sup>7</sup>.

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The data used in the preparation of this article were obtained from the Prognostic and Natural History Study (PNHS), provided by the Amyloid Imaging to Prevent Alzheimer's Disease Consortium (AMY-PAD). As such, investigators within the AMYPAD PNHS and AMYPAD Consortium contributed to the design and implementation of AMYPAD and/or provided data but did not participate in the analysis or writing of this report. We acknowledge Eugenio Rosado and Michael K. James (Grifols) for their editorial assistance in the preparation of this manuscript.

#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.tjpad.2025.100465](https://doi.org/10.1016/j.tjpad.2025.100465).

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